Biochemical Society Transactions (2025) **53** 267–280
https://doi.org/10.1042/BST20240507

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Review Article

Conformational dynamics of the nuclear pore complex central channel

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The nuclear pore complex (NPC) is a vital regulator of molecular transport between the nucleus and cytoplasm in eukaryotic cells. At the heart of the NPC's function are intrinsically disordered phenylalanine-glycine-rich nucleoporins (FG-Nups), which form a dynamic permeability barrier within the central channel. This disordered nature facilitates efficient nucleocytoplasmic transport but also poses significant challenges to its characterization, especially within the nano-confined environment of the NPC. Recent advances in experimental techniques, such as cryo-electron microscopy, atomic force microscopy, fluorescence microscopy, and nuclear magnetic resonance, along with computational modeling, have illuminated the conformational flexibility of FG-Nups, which underpins their functional versatility. This review synthesizes these advancements, emphasizing how disruptions in FG-Nup behavior—caused by mutations or pathological interactions—contribute to diseases such as neurodegenerative disorders, aging-related decline, and viral infections. Despite progress, challenges persist in deciphering FG-Nup dynamics within the crowded and complex cellular environment, especially under pathological conditions. Addressing these gaps is critical for advancing therapeutic strategies targeting NPC dysfunction in disease progression.

Introduction

The nuclear pore complex (NPC) is a critical structure in eukaryotic cells that enables the selective exchange of molecules between the nucleus and cytoplasm. This large, multi-protein assembly spans the nuclear envelope and comprises approximately 30 distinct proteins, collectively known as nucleoporins (Nups). These Nups are organized into several key substructures, including the inner pore ring, nuclear ring, cytoplasmic ring, nuclear basket, and cytoplasmic filaments (Figure 1) [1–7]. At the core of the NPC's function are the intrinsically disordered, phenylalanine-glycine-rich nucleoporins (FG-Nups), which reside in the central channel and form a selective permeability barrier. This barrier regulates the passive diffusion of small molecules and facilitates the active transport of larger macromolecules in coordination with nuclear transport receptors (NTRs) [8–10].

The NPC's ability to maintain both high selectivity and rapid transport has led to diverse models explaining the mechanisms underlying its permeability barrier. FG-Nups have been proposed to act as polymer brushes, entropic gates, or liquid-like and gel-like phases [11–19]. Weis evocatively described FG-Nups as behaving like 'oily spaghetti' or a 'gummy bear,' emphasizing their dual nature as flexible, disordered entities and cohesive networks [20]. The function of FG-Nups is inherently tied to their conformational dynamics, which are determined not only by their amino acid sequences but also by the specific cellular environment, where various factors, such as molecular crowding, ionic strength, and interactions with NTRs and cargo molecules, play pivotal roles. However, the disordered nature of FG-Nups poses challenges in examining their conformations, especially within the unique nano-confined environment of the NPC. This review provides an overview of recent advances in experimental and computational approaches for studying FG-Nups. It also explores how the microenvironment of the central channel shapes FG-Nup dynamics and highlights the implications for NPC function in health and disease, particularly in the contexts of neurodegenerative disorders and viral infections.

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Received: 20 September 2024
Revised: 2 December 2024
Accepted: 12 December 2024

Version of Record
Published: 7 February 2025

Biochemical Society Transactions (2025) 53 267–280
https://doi.org/10.1042/BST20240507

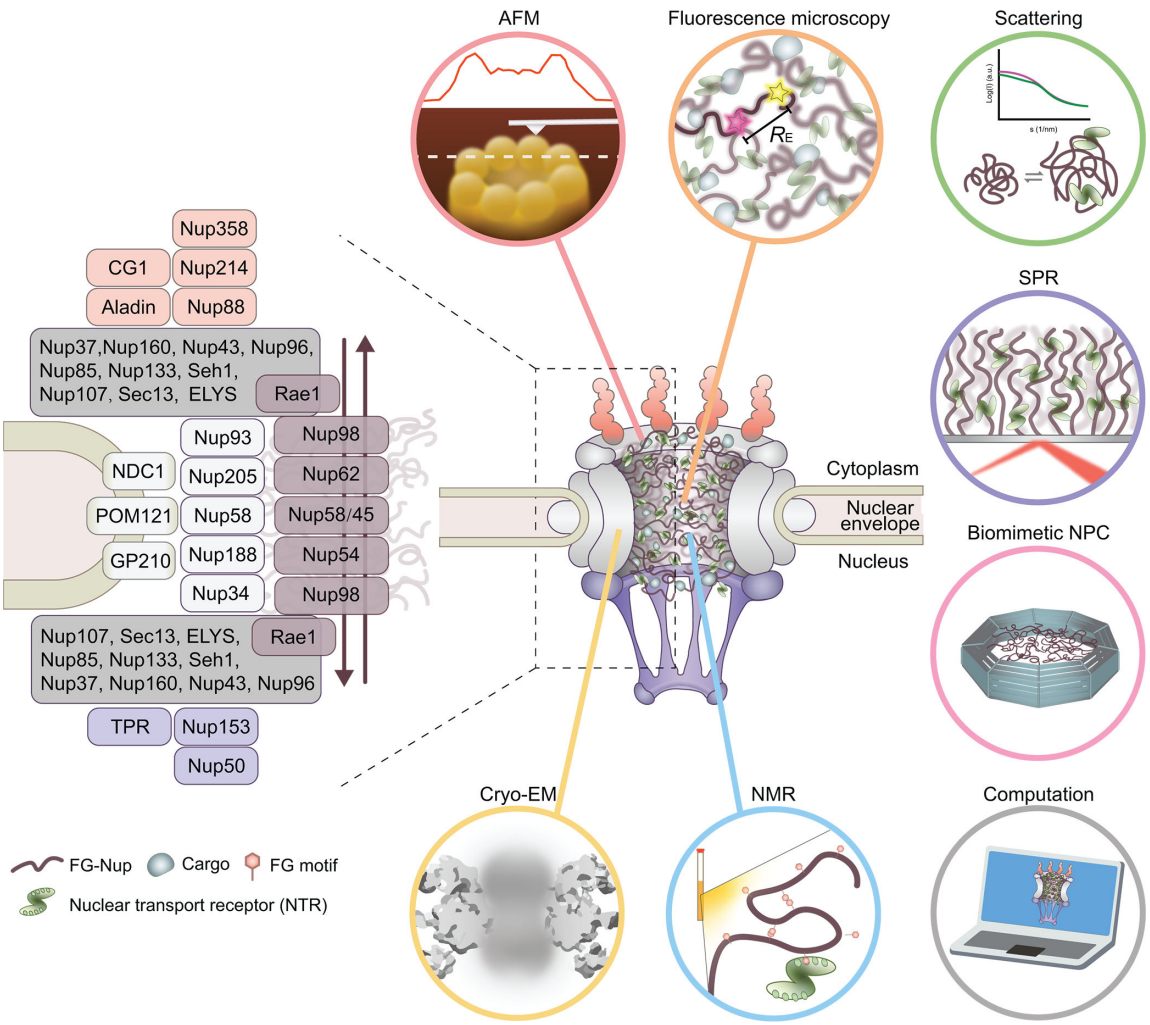

Figure 1: Techniques for studying the conformational dynamics of the nuclear pore complex (NPC) central channel.

Left: Schematic illustration of the structure and composition of the NPC. Right: Overview of various techniques used to explore the conformational dynamics of the NPC central channel, including atomic force microscopy (AFM), fluorescence microscopy, scattering techniques, surface plasmon resonance (SPR), biomimetic NPC, computational methods, nuclear magnetic resonance (NMR), and cryo-electron microscopy (cryo-EM). The connecting lines indicate that these methods allow for *in situ* measurements of the NPC.

Methods to resolve the conformations and function of the NPC

Cryo-electron microscopy/tomography

Advances in cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET) have revolutionized the study of NPC by providing unprecedented insights into the intricate scaffold architecture [21–24]. Cryo-EM has been particularly transformative in obtaining near-atomic resolution images of isolated proteins and NPC subcomplexes *in vitro*, uncovering key interactions between core scaffolding Nups [25,26]. On the other hand, cryo-ET excels in visualizing the NPC architecture within intact cellular environments [4,27–29]. Recent cryo-ET studies have revealed how NPCs can adapt their structure to accommodate different physiological conditions, particularly the expansion and contraction of the central channel, highlighting the role of linker proteins in maintaining both the structural integrity and dynamic plasticity of the complex [22,30,31]. Furthermore, recent integration of artificial intelligence (AI)-based models with cryo-ET data has further refined our understanding of NPC dynamics, enabling the prediction of various conformational states under different cellular conditions [32].

Despite these advancements, cryo-EM and cryo-ET primarily offer static snapshots of NPC structures, making it challenging to capture dynamic processes in real time, particularly the behavior of intrinsically disordered FG-Nups. The dense, blurred masses often observed in cryo-EM images hint at the underlying conformational flexibility of FG-Nups in the central channel, but capturing these dynamic processes requires complementary approaches [24,31].

Atomic force microscopy

Atomic force microscopy (AFM) offers nanoscale insights into the mechanical properties and flexibility of NPC components under near-physiological conditions, providing complementary information to structural imaging techniques [33,34]. Recent advances in high-speed AFM (HS-AFM) have further improved the temporal resolution, allowing for real-time observation of dynamic fluctuations of FG-Nups between extended, retracted, and entangled states [35]. These observations are crucial for understanding the structural plasticity of FG-Nups and their role in modulating the selective permeability barrier of the NPC. In addition, by capturing the real-time interactions between FG-Nups and NTRs, AFM has offered new insights into the transient binding events that facilitate transport through the NPC [36,37].

AFM focuses primarily on surface-level structures and interactions, where both the cytoplasmic and nuclear sides of the NPC have been probed [38]. With high speed and high-resolution scales, the visualization of the assembly and dynamics on the surface of the NPC becomes feasible. However, certain aspects of the NPC's internal dynamics, particularly in the deeper regions of the channel, remain beyond its reach.

Surface plasmon resonance

Surface plasmon resonance (SPR) is a powerful technique for studying real-time, label-free interactions between biomolecules, including those within the NPC. By measuring changes in refractive index at a sensor surface, SPR provides kinetic data such as binding affinities, interaction stoichiometries, and association/dissociation rates [39]. SPR has been particularly useful in characterizing the interactions between FG-Nups and key NTRs, helping to elucidate the molecular mechanisms by which selective transport occurs through the central channel [40,41]. SPR has also been applied to study pathological conditions involving the NPC, such as neurodegenerative diseases. For instance, phosphorylation was identified as a key regulatory mechanism driving the aggregation of Tau and Nup98, which could disrupt NPC function by impeding transport through the pore [42].

SPR studies are typically conducted *in vitro* with purified components, and immobilization of binding partners on the sensor surface may introduce artifacts. While SPR is highly effective at probing interaction kinetics, it is best used alongside other techniques to gain deeper insights into the conformational changes that occur during these interactions.

Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) spectroscopy provides atomic-level insights into the structure and dynamics of intrinsically disordered proteins like FG-Nups. It is particularly useful for studying transient interactions between FG-Nups and NTRs, shedding light on the molecular mechanisms underlying selective permeability and cargo transport through the NPC [43,44]. Recent advancements in in-cell NMR have enabled the study of protein behavior directly within living cells, providing a more physiologically relevant perspective on FG-Nup function. For example, in *Saccharomyces cerevisiae*, in-cell NMR of an FG-Nup fragment (FSFG-K) revealed that it remains fully disordered *in vivo*, with no evidence of secondary structure [45]. In contrast, *in vitro* NMR studies combined with cryo-EM have demonstrated that FG-Nup98 can form amyloid-like aggregates under specific conditions, suggesting a potential mechanism for regulating the NPC's permeability barrier and the involvement of FG-Nups in neurodegenerative diseases [46,47]. These differing results may stem from variations in the FG motifs examined or the inability of *in vitro* systems to accurately reflect the native cellular environment.

While in-cell NMR provides a more physiologically relevant approach, it still faces challenges, such as low signal-to-noise ratios due to the crowded intracellular environment and difficulties with isotopic labeling under natural conditions. Despite these hurdles, in-cell NMR continues to develop, offering the potential for deeper insights into real-time protein dynamics within the NPC central channel.

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https://doi.org/10.1042/BST20240507

Scattering techniques

Scattering techniques, including small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), and dynamic light scattering (DLS), provide valuable information on the size, shape, and structural organization of proteins, especially disordered ones like FG-Nups, in solution [48,49]. SAXS is particularly useful for studying the extended or collapsed conformations of FG-Nups, providing insights into how their structure changes in response to environmental conditions [25,50]. SANS, with its sensitivity to differences in neutron scattering length densities, can differentiate between components within larger complexes, making it ideal for studying the interactions between FG-Nups and NTRs [51]. DLS, on the other hand, measures the diffusion rates of particles in solution, providing data on the hydrodynamic radius and overall size distribution of FG-Nups or their complexes [43].

Due to the limitation in resolving atomic-level details, scattering data are often integrated with higher resolution techniques such as cryo-EM or NMR to gain a more complete understanding of FG-Nup behavior and NPC function. Nevertheless, SAXS, SANS, and DLS remain essential tools for investigating the structural dynamics of FG-Nups under near-native conditions.

Fluorescence microscopy

Fluorescence microscopy is a versatile and essential tool for visualizing the NPC in real time. For example, by tagging proteins with fluorescent labels, researchers can visualize nucleocytoplasmic transport processes of large cargos in the cell [52]. Additionally, fluorescence correlation spectroscopy (FCS) has enabled the tracking of fluorescently tagged Nups during NPC assembly in dividing cells, helping to build a spatiotemporal model of the NPC assembly pathway [53].

Super-resolution fluorescence microscopy has proven especially valuable for resolving NPC components at the nanoscale within single cells [54,55]. When combined with computational classification for single-NPC analysis, this technique has uncovered structural heterogeneity of the nuclear ring and the nuclear basket [56]. It has also been employed to track the movement of cargo molecules through the NPC, providing detailed insights into cargo-complex distribution along the central channel [57–59].

Recent advances, including minimal fluorescent tag labeling via genetic code expansion and fluorescence lifetime imaging of fluorescence resonance energy transfer (FLIM-FRET), have enabled us to directly probe the conformations of FG-Nups within their native cellular environments [60]. We found that the FG domain of Nup98 adopts much more expanded conformations *in situ* compared with the highly collapsed state of single chains in solution, thereby facilitating nuclear transport. Our work provides valuable molecular insights into the conformational changes and interactions of FG-Nups in their functional state. Such *in situ* analysis is crucial for understanding how FG-Nups contribute to the NPC's selective permeability and how their dynamic conformations drive nucleocytoplasmic transport.

Biomimetic NPC

Biomimetic NPC provides nanometer-scale control over biomolecular positioning, allowing investigation of Nup conformation and function in simplified, yet functional systems [61–63]. For instance, DNA origami techniques have been employed to create biomimetic platforms, such as Nups organized on DNA (NuPOD), which enable the analysis of Nup dynamics, selectivity, and interaction with cargo molecules combining techniques like DLS, AFM, and cryo-EM [34,62,64,65]. Those biomimetic systems have demonstrated that FG-Nups can form distinct permeability barriers influenced by the spatial arrangement and density of FG motifs, which, in turn, affect cargo transport selectivity [61,66–68]. Biomimetic models have also been used to mimic pathological processes, such as nuclear entry of human immunodeficiency virus (HIV), elucidating how Nup358 and Nup153 create affinity gradients for viral capsid transit [69].

While biomimetic systems provide invaluable insights into NPC function, they remain simplified approximations of the native NPC, lacking the complexity of the intracellular environment. Future advancements in biomimetic design, incorporating additional layers of complexity, will help bridge the gap between *in vitro* models and the native cellular context.

Computational methods

Computational approaches have emerged as powerful tools for predicting NPC structures, simulating dynamics, and exploring molecular interactions within the central channel. These methods allow

researchers to model the conformational flexibility and interactions of FG-Nups, providing insights that complement experimental findings [32,60,70–72]. Recent breakthroughs, such as AI-based structure prediction tools, have enabled the generation of high-resolution NPC models of its scaffold dynamics [32,73].

Molecular dynamics simulations have proven particularly valuable in elucidating how the rapid, multivalent interactions between FG motifs and NTRs drive selective transport [74–76]. Computational models have also explored how variations in FG-Nup sequence or post-translational modifications (PTMs), such as phosphorylation, influence NPC selectivity by altering hydrophobic, electrostatic, or steric interactions within the channel [65,77–80]. Importantly, computational modeling has provided mechanistic insights into bidirectional transport within the NPC [52,77,80–82].

While computational methods offer unparalleled flexibility and detail, their accuracy heavily depends on the quality of the underlying force fields and experimental data used for model validation. Nonetheless, as computational power and modeling techniques continue to improve, these methods will become increasingly indispensable for understanding NPC dynamics and function, particularly when combined with experimental results.

Integrative structural biology approaches

The central channel of the NPC is a highly dynamic and complex environment, making it challenging to study using any single method (see Table 1 for the comparison of the techniques discussed above). Integrative structural biology has established itself as a powerful framework to address this challenge [72]. By synthesizing information from various experimental and computational methods, this approach can provide an atomic-to-mesoscale view of the NPC's architecture and dynamic behavior. Recent studies have utilized integrative approaches to resolve both the core structure and peripheral regions of the NPC, including the nuclear basket, which plays critical roles in mRNA transport and chromatin organization [29,70,72]. Coarse-grained Brownian dynamics simulations, coupled with spatiotemporal modeling, have further elucidated how FG-Nups form selective permeability barriers and mediate transport [83].

The strength of integrative approaches lies in their ability to incorporate diverse datasets, resolving inconsistencies and filling gaps in individual methods. However, challenges persist in modeling the NPC's dynamic behavior within the crowded intracellular environment and in reconciling sparse or noisy datasets. Future advancements in integrative modeling, particularly through the inclusion of machine learning and real-time cellular imaging, hold great promise for further unraveling the NPC's structural dynamics and its role in cellular processes.

Dynamics, interactions, and modifications of FG-Nups

Functional characteristics and dynamics of FG-Nups

Most FG-Nups reside within the NPC central channel, while some extend into the nucleoplasm and cytoplasm [84]. FG-Nups are categorized by their FG motifs, such as FxFG, GLFG, and xxFG [85]. Studies in yeast have shown that even when more than half of the FG-Nup domains are deleted, the permeability barrier remains intact, suggesting functional redundancy [86]. On the other hand, specific FG domains are indispensable for maintaining selective permeability, as their absence can lead to inappropriate diffusion and NPC 'leakage' [87]. While FG-Nup primary sequences evolve rapidly, their intrinsic disorder and hydrophilic motifs are conserved, maintaining essential functional properties [88–90]. However, the absence of a prokaryotic ancestor for FG-Nups complicates the understanding of their evolutionary origins [91].

FG-Nups interact with scaffold Nups, playing a critical, yet not fully understood, role in maintaining scaffold architecture and organizing the permeability barrier [53,92,93]. One key unresolved question is how FG-Nup conformations dynamically adapt to the contraction and dilation of the NPC scaffold, a process essential for its function [30].

The functional dynamics of FG-Nups, particularly their role in forming the selective permeability barrier, have attracted significant attention. The nature of this barrier is influenced by whether FG-Nups exhibit cohesive (self-interacting), repulsive behavior, or a combination of both [84,94–98]. Several models and approaches have been developed to study their behavior. FG-Nups have been observed to transition between collapsed states and extended conformations in different experimental conditions

Table 1: Comparison of techniques for studying the conformational dynamics of the NPC central channel

Method	Resolution	Compatibility with live cells	Ability to study dynamics	Time scale of observation	Advantages	Limitations
Cryo-electron microscopy/tomography (Cryo-EM/ET) [4,21–26,28–32]	Near-atomic	Limited (*in vitro* or *ex vivo* samples)	Limited (static snapshots)	Milliseconds (via rapid freezing) for capturing static states	High-resolution structural data, ideal for scaffold visualization	Static images, difficult to capture dynamic processes
Atomic force microscopy (AFM) [33–38]	Nanoscale	Limited (permeabilized cells or isolated NPCs)	Yes (real-time for surface components)	Milliseconds to seconds	Real-time mechanical measurements, near-physiological conditions	Limited to surface interactions, requires isolated samples
Surface plasmon resonance (SPR) [39–42]	Sub-micrometer	No	Yes (real-time interaction kinetics)	Seconds to minutes	Detailed kinetic data, binding affinities, and stoichiometry	*In vitro* only, potential artifacts from immobilization
Nuclear magnetic resonance (NMR) [43–47]	Atomic	Yes (in-cell NMR possible)	Yes (protein flexibility and interactions)	Milliseconds to seconds	Atomic-level details, ideal for studying disordered proteins like FG-Nups	Challenging signal-to-noise ratio in cells, complex sample preparation
Small-angle X-ray/neutron scattering (SAXS/SANS) [25,43,48–51]	Low to moderate	No	Moderate (overall shape, not detailed)	Minutes to hours	Captures structural organization in solution	Limited resolution, lacks atomic detail
Fluorescence microscopy (including FLIM-FRET, super-resolution) [52–60]	Moderate (diffraction-limited) to nanoscale (super-resolution)	Yes	Yes (real-time observation of FG-Nup dynamics)	Milliseconds to seconds	Real-time dynamics in living cells, wide applicability to various systems	Photobleaching, lower resolution compared with electron microscopy
Biomimetic NPC [34,61–69]	Method-dependent	No	Yes (reconstituted dynamics)	Method-dependent	Allows reconstitution of NPC components and their interactions	System complexity may not fully replicate native NPC environment
Computational methods [32,70–82]	Atomic to mesoscale	*In silico* simulations	Yes (molecular and mesoscopic dynamics)	Picoseconds to seconds	Detailed molecular insights into conformational dynamics, molecular transport, and environmental influences.	Relies on approximations and assumptions; requires validation with experimental data.

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Biochemical Society Transactions (2025) **53** 267–280
https://doi.org/10.1042/BST20240507

[40,60,84,95,99,100]. Additionally, FG-Nups can assemble into supramolecular structures, such as liquid-like condensates, solid-like hydrogels, or amyloid fibers, which potentially linked to neurodegenerative diseases, as discussed below in Section *Pathological consequences of NPC dysfunction* [88,101,102]. A consensus on FG-Nup behavior has yet to be reached, largely due to their dynamic and disordered nature, highlighting the need for further research to fully understand how their conformational states contribute to the NPC's selective permeability barrier within the cellular environment.

Interaction between FG-Nups and NTRs

The NPC central channel contains not only FG-Nups but also numerous NTRs and cargos, which together account for the majority of its mass [103]. Key NTRs include karyopherins (Kaps), NTF2, and Mex67-Mtr2 (NXF1-NXT1 in metazoans). The Kap family is responsible for transporting most proteins and diverse RNAs, including rRNAs, tRNAs, miRNAs, and snRNAs [104]. NTF2 facilitates the nuclear import of RanGDP, where Ran functions as a molecular switch, regulating the assembly and disassembly of transport complexes and directing Kap-mediated nucleocytoplasmic transport [105]. Furthermore, Mex67-Mtr2 (NXF1-NXT1) mediates the export of large amounts of mRNA, highlighting the broad functional diversity of NTR-mediated transport [106,107].

FG-Nup interactions with NTRs are pivotal for efficient transport. Crystallography has identified hydrophobic FG-binding pockets in NTRs like importin-β, which bind FxFG peptides [108]. Advanced methods, such as HS-AFM, reveal distinct binding dynamics between FG-Nups and various NTRs [36,109]. These interactions, driven by low-affinity, multivalent contacts between FG-Nup and NTRs, are essential for efficient and rapid transport through the NPC [108,110–113]. Mutations that enhance NTR affinity for FG repeats, such as NTF2-N77Y, can disrupt nucleocytoplasmic transport, highlighting the need for fast, low-affinity interactions [114].

Super-resolved 3D tracking of cargo transport through the NPC has identified distinct NTR pathways for different cargo complexes [57]. Remarkably, recent simulations suggest that NTR crowding may enhance transport efficiency rather than impede it, proposing a self-regulatory mechanism for cargo density and flux [81]. Future experiments could track cargo transport bidirectionally to determine if distinct transport pathways exist and to verify this proposed clogging-free mechanism.

PTMs of FG-Nups

PTMs significantly modulate FG-Nup function by altering their interactions with NTRs and their biophysical properties. Among these, O-linked glycosylation has been extensively studied. Although glycosylation does not affect the correct localization of FG-Nups within the NPC [115], it can influence transporter function through O-linked N-acetylglucosamine (O-GlcNAc) residues [116]. This modification can influence the phosphorylation state of glycosylated Nups and serve as binding sites for lectin-mediated transport [117]. O-GlcNAc, in particular, plays a role in maintaining the structural integrity of the NPC and regulating nucleocytoplasmic transport functions [118,119].

Phosphorylation of FG-Nups is another key modification. Simulations have shown that phosphorylation reduces inter-residue attraction, resulting in the extension of FG-Nups and a less dense FG network, which increases diffusion rates for inert molecules but reduces NPC selectivity [79]. Furthermore, phosphorylation of FG-Nups by extracellular signal-regulated kinase (ERK) can inhibit their interaction with NTRs, modulating the permeability barrier properties of the NPC without altering its overall structure [120].

In addition, ubiquitination contributes to regulating FG-Nup function. More than half of the NPC proteins have been found to be conjugated to ubiquitin. For instance, Nup159, located exclusively on the cytoplasmic side of the NPC, is monoubiquitinated by the Cdc34/SCF enzyme complex. Interestingly, blocking this modification does not affect nuclear transport or the organization of the NPC [121].

A major challenge in studying FG-Nups *in vitro* is the difficulty of reproducing their PTMs, which are context-dependent, dynamic, and shaped by the complex native environment of the central channel. This limitation often leads to discrepancies between *in vitro* and intracellular observations. Investigating FG-Nups under native conditions is, therefore, essential for a comprehensive understanding of their role in NPC function.

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https://doi.org/10.1042/BST20240507

Pathological consequences of NPC dysfunction

The conformational flexibility of FG-Nups is vital for NPC selectivity and transport regulation. Disruptions in FG-Nups or their interactions with other proteins can result in profound pathological consequences. NPC dysfunction is increasingly implicated in neurodegenerative diseases, aging, and viral infections.

The deterioration of NPC function has been increasingly linked to neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and Alzheimer's disease (AD) [122,123]. In these conditions, NPC disruption impairs nucleocytoplasmic transport and cellular homeostasis, contributing to the pathogenesis. FG-Nups often become mislocalized or aggregated, leading to impaired transport and the accumulation of toxic proteins in the cytoplasm or nucleus. For instance, studies have shown that in ALS/FTD, mutations in proteins such as C9orf72 or TDP-43 are associated with the mislocalization of specific Nups, including Nup62 and POM121, leading to a breakdown in nucleocytoplasmic transport [124,125]. In AD, tau pathology is also associated with NPC dysfunction. Hyperphosphorylated tau binds to FG-Nups such as Nup98, resulting in their mislocalization and aggregation [42,126]. This interaction disrupts the permeability barrier of the NPC and interferes with the selective transport of essential molecules, further promoting neurodegeneration.

The integrity of the NPC also declines with age, contributing to age-related dysfunction in non-dividing cells like neurons and cardiomyocytes [127]. This age-dependent deterioration of NPCs has been linked to impaired nucleocytoplasmic transport, leading to the accumulation of damaged proteins and RNA within the nucleus, ultimately contributing to cellular senescence and tissue degeneration [128]. Moreover, age-related reductions in Nsp1, a key FG-Nup, have been shown to mimic NPC aging phenotypes by disrupting the phase state of FG-Nups, shifting them from a liquid-like state to a more aggregated state [129].

Viral pathogens also exploit the NPC for nuclear entry [130,131]. For example, HIV directly interacts with FG-Nups which may facilitate the import of its capsid into the nucleus [69,132,133]. This interaction could disrupt the normal permeability barrier of the NPC, allowing the viral capsid to bypass selective transport mechanisms that would otherwise prevent such large structures from entering the nucleus. Despite ongoing research, the precise mechanisms by which the HIV capsid protein interacts with FG-Nups remain unclear. This highlights a critical gap in our understanding of how NPC dynamics can be manipulated by pathogens, with potential implications for antiviral therapies.

Concluding remarks

The conformational dynamics of the NPC central channel, particularly the behavior of FG-Nups, are fundamental to understanding nucleocytoplasmic transport. The functional role of FG-Nups is linked to their conformational dynamics, which are influenced by their interactions with other FG-Nups, NTRs, cargo molecules, and PTMs. These intrinsically disordered proteins exhibit remarkable environmental sensitivity, underscoring the need to study them in their native cellular context to fully grasp their functional significance [60].

Despite significant advances, capturing the dynamic nature of FG-Nups remains a formidable challenge due to their transient interactions and inherent structural flexibility, which are not easily resolved by any single experimental technique. Addressing this complexity requires a multidisciplinary approach —integrating cryo-EM, AFM, NMR, scattering techniques, biomimetic NPC systems, computational modeling, and advanced fluorescence microscopy. The synergy of these structural, biophysical, and functional methodologies will provide a more comprehensive understanding of how NPCs maintain selective permeability and facilitate efficient molecular transport.

Looking ahead, future research should prioritize intracellular studies of FG-Nups, focusing on how these proteins behave in the crowded, complex environment of living cells. Key questions remain about how FG-Nups transition between expanded and collapsed states, adapt to scaffold dynamics, and respond to pathological disruptions (Figure 2). Investigating FG-Nup dynamics under both physiological and pathological conditions, such as in neurodegenerative diseases and viral infections, will be crucial for deepening our understanding of NPC function and identifying new therapeutic targets.

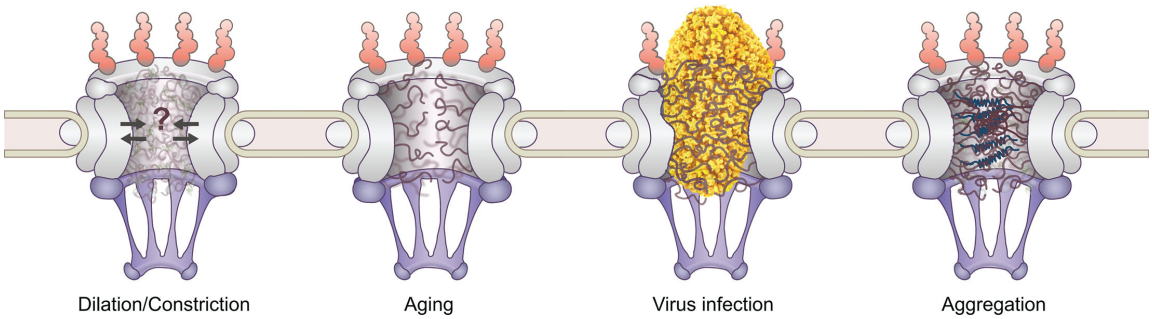

Figure 2: Key open questions in the field.

(1) How do FG-Nups change conformation—becoming more expanded or collapsed—during dilation or constriction of the NPC central channel? (2) In age-dependent deterioration of the NPC, how do the conformation and dynamics of FG-Nups change, and how is this linked to impaired nucleocytoplasmic transport? (3) During viral infections, such as HIV, do FG-Nups expand or collapse? (4) When neurodegenerative disease-linked proteins, such as Tau, aggregate in the central channel, how do FG-Nup dynamics change?

Perspectives

- The NPC is crucial for maintaining cellular homeostasis by regulating the exchange of molecules between the nucleus and cytoplasm. Dysfunction in NPCs is increasingly recognized as a contributing factor to a variety of diseases, including neurodegenerative disorders, age-related decline, and viral infections. Understanding the molecular mechanisms governing NPC function is, therefore, essential for addressing these health challenges.
- Significant strides have been made in understanding the structural and functional dynamics of FG-Nups, the key components forming the NPC's selective permeability barrier. Advances in techniques, such as cryo-EM, NMR, AFM, biomimetic NPC, scattering techniques, computational method, and advanced fluorescence microscopy, have illuminated aspects of FG-Nup behavior, but challenges remain in fully capturing their conformational states in native cellular environments.
- Future research should focus on real-time, in-cell studies of FG-Nups using advanced imaging technologies and other biophysical techniques. Uncovering how FG-Nups dynamically regulate NPC function in both healthy and diseased states will be pivotal for identifying novel therapeutic strategies aimed at targeting NPC dysfunction, particularly in neurodegenerative diseases and viral infections.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Funding

M.Y. acknowledges funding support by the National Natural Science Foundation of China (Project No. 32471521).

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CRediT Author Contribution

Conceptualization: M.Y.; Original Draft Preparation: C.Y., G.Z., and M.Y.; Review and Editing: C.Y., G.Z., and M.Y. All authors have read and approved the final version of the manuscript.

Biochemical Society Transactions (2025) **53** 267–280
https://doi.org/10.1042/BST20240507

Acknowledgments

The authors thank all the members of the Yu laboratory for constructive discussions.

Abbreviations

AD, Alzheimer's disease; AFM, Atomic force microscopy; AI, Artificial intelligence; ALS, Amyotrophic lateral sclerosis; Cryo-EM, Cryo-electron microscopy; Cryo-ET, Cryo-electron tomography; DLS, Dynamic light scattering; ERK, extracellular signal-regulated kinase; FCS, Fluorescence correlation spectroscopy; FG-Nups, Phenylalanine-glycine-rich nucleoporins; FLIM-FRET, Fluorescence lifetime imaging of fluorescence resonance energy transfer; FTD, Frontotemporal dementia; HIV, Human immunodeficiency virus; HS-AFM, High-speed atomic force microscopy; Kaps, Karyopherins; NMR, Nuclear magnetic resonance; NPC, Nuclear pore complex; NTRs, Nuclear transport receptors; NuPOD, Nups organized on DNA; Nups, Nucleoporins; O-GlcNAc, O-linked N-acetylglucosamine; PTMs, Post-translational modifications; SANS, Small-angle neutron scattering; SAXS, Small-angle X-ray scattering; SPR, Surface plasmon resonance.

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