University of Groningen

Sol-gel transition in solutions of FG-Nups of the nuclear pore complex
Ghavami, Ali; Van der Giessen, Erik; Onck, Patrick R.

Published in:
Extreme Mechanics Letters

DOI:
10.1016/j.eml.2018.04.006

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 08-10-2023
The nuclear pore complex (NPC) is a giant molecular gate that regulates the transport of molecules and macromolecules across the nuclear envelope in a selective and directional manner. Using a coarse-grained one-bead-per-amino-acid model and percolation analysis we have characterized the gel formation of solutions of FG-Nups that line the central channel of the NPC. We show that FG-Nup solutions can indeed form a percolated network (gel) when the concentration of the Nups exceeds a certain limit. Furthermore, we show that this critical concentration depends on the amino acid composition of the FG-Nups and correlates with the ratio between charged and hydrophobic amino acids. Comparison with the protein concentrations inside the yeast NPC reveals that it is very likely that the FG-Nups form a gel phase.

**1. Introduction**

Nuclear pore complexes (NPCs) are highly selective gates which regulate the transport of molecules across the nuclear envelope [1–4]. The NPC allows for passive transport of small molecules and provides facilitated transport of large macromolecules through binding with nuclear transport receptors (NTRs). The transport channel of the NPC is lined with several copies of disordered proteins that are rich in (F) and (G) repeats (FG-Nups). The FG-Nups which have a very heterogeneous amino acid sequence with charged and hydrophobic regions [5–7]. The collective state of these disordered proteins in the nuclear pore complex is notoriously hard to probe in vivo. In vitro it has been shown that FG-Nup solutions can form polymer-brushes [8–11] as well as cross-linked hydrogels [12–14], both with NPC-like transport features. At the same time, there are studies that suggest that both the brush and the gel state coexist in the NPC [15]. Hence, there is no consensus on the collective state of the FG-Nups in the NPC.

The gel formation of FG-Nups was first reported for a solution of Nsp1 from yeast where the transition from a solution state (sol) to a gel-state (gel) had been triggered by lowering the pH of the solution to the physiological pH [12]. It was shown that an elastic gel is formed at densities higher than 8 mg/mL and that FG hydrogels with a sufficient concentration of FG-repeat domains form an effective barrier against inert proteins larger than the permeability limit of the NPC. On the other hand, NTR-cargo complexes with a similar size were found to permeate 1000 times faster into the gel [1,16], clearly showing that the hydrogels are endowed with a selective permeability barrier. These findings suggest that the gel state can be a viable state of the FG-Nups in the transfer channel of the NPC.

It has been shown that the formation of FG hydrogels requires many cohesive units to stabilize the gel [17], which has been examined through a mutational analysis in which the F to S mutated Nsp1p was unable to form an elastic hydrogel even at high concentrations, while the F to Y mutation did not considerably alter the gel formation propensity [12]. Further experimental and theoretical investigations indicated that the nature of these interactions are mainly hydrophobic [17,18].

Supplementary to other computational investigations of the nuclear pore complex [19–22], we have previously studied the distribution of the FG-Nups inside the NPC through a one-bead-per-amino-acid (1BPA) coarse grained molecular dynamics model [23,24]. Our simulation results revealed a low density region inside a 10 nm core of the NPC surrounded by a high density, donut-like region, rich in FG-repeats. Furthermore, we used the model to characterize the energy barrier of the NPC during active and passive transport and demonstrated that the presence of hydrophobic patches on the surface of the cargos can significantly reduce the transport barrier [25].

In the present study, we aim to answer the question whether the characteristic densities in these studies (i.e. in the center of the pore and in the high density donut region) give rise to a sol–gel transition or not. To do so, we will investigate the gel formation properties of different homogeneous FG-Nup solutions using our 1BPA coarse-grained model (see Ref. [24]). We use percolation theory to identify the sol–gel transition of FG-Nup solutions at different concentrations. Furthermore, we study the effect of FG-repeats on the gelation of FG-Nups by mutating the F residues to other hydrophobic or to hydrophilic residues. In addition, the critical concentration at which the sol–gel transition occurs is
characterized as a function of the charge to hydrophobicity ratio of the amino acid sequence of the FG-Nups. We conclude with a discussion on the probability of gel formation to occur inside the core of the yeast NPC.

2. Method

2.1. Coarse-grained one-bead-per-amino-acid (1BPA) model

In this section we will first briefly summarize the 1BPA molecular dynamics method that we have developed to model the intrinsically disordered FG-Nups of the NPC. For details the reader is referred to [23,24]. Details on the percolation analysis and the simulation set-up are given in Sections 2.2 and 2.3. Subsequently, in Section 3, we will apply the method to study the sol–gel transition in a selection of FG-Nup systems.

In the 1BPA model each amino acid is represented by one bead with an average mass of 120 Da [24]. The distance between neighboring beads is fixed using a stiff harmonic potential \( \phi_{\text{bond}} = k_b(r−b)^2 \) with \( k_b = 8038 \) kJ mol\(^{-1}\) and \( b = 0.38 \) nm. The torsion and bending potentials are obtained from the Ramachandran data of the coiled regions of protein structures [23]. They are given in full detail in [23] and will not be repeated here. Since FG-Nups lack any stable secondary structure [26,27,5,28], hydrogen bonding is not incorporated in our model. The solvent molecules are not modeled explicitly. Instead, the solvent-residue interactions are implicitly accounted for through hydrophobic and hydrophilic interactions between the amino acid residues. In order to take into account both attractive hydrophobic and repulsive hydrophilic interactions, the following potential is used:

\[
\phi_{\text{hp}}(r) = \begin{cases} 
\varepsilon_{\text{hp}} \left( \frac{1}{r} \right)^{8} - \varepsilon_{ij} \left( \frac{4}{3} \left( \frac{1}{r} \right)^{6} - \frac{1}{3} \right) & r \leq \sigma \\
(\varepsilon_{\text{hp}} - \varepsilon_{ij}) \left( \frac{1}{r} \right)^{8} - \sigma & \sigma \leq r,
\end{cases}
\]

where \( \varepsilon_{ij} = \varepsilon_{\text{hp}} \left( \frac{1}{r} \right)^{8} \) is the strength of the interaction for each pair of amino acids (i, j) and \( \sigma = 0.60 \) nm is the average residue diameter [29]. The hydrophobicity scales obtained from partition energy measurements [30–32], are normalized between 0 and 1 and the average value of the experimental data is used to define the relative hydrophobic strength for each amino acid \( \varepsilon_i \in [0, 1] \) (see SI in [24]). The \( \varepsilon_{\text{hp}} \) can be interpreted as the absolute hydrophobic strength between the most hydrophobic amino acids, while \( \varepsilon_{\text{hp}} \) defines the intensity of the repulsive hydrophilic interactions. To account for the electrostatic interactions and screening effect of polar water molecules and free ions, we use the modified Coulomb law

\[
\phi_{el} = \frac{q_i q_j}{4\pi\varepsilon_0 \varepsilon_r \left( \frac{1}{r} \right)} \exp(-\kappa r).
\]

The solvent dielectric constant is implemented through a sigmoidal function \( \varepsilon_i(r) = S_d \left[ 1 - e^{\frac{r - z}{\varepsilon_r \left( \frac{1}{r} \right)}} \right]\), where \( S_d = 80, z = 0.25 \) nm [33,34] and the Debye screening coefficient is set to \( \kappa = 1.0 \) nm\(^{-1}\), similar to that of the cytoplasm [35].

While parameterizing the model, first the \( \varepsilon_{\text{rep}} \) and \( \varepsilon_{\text{hp}} \) were set to 10.0 and 13.0 kJ mol\(^{-1}\) in order to account for the contact energy of the most hydrophobic residues, i.e., \(-5.2 \) kJ mol\(^{-1}\), as suggested in [36]. In the next step, the exponent \( \alpha = 0.27 \) was selected in order to reproduce the experimentally-obtained Stokes radius of a low-charge FG domain. In addition, the hydrophobic strength of three charged residues (i.e., D, E and K) was adjusted to reproduce the Stokes radius of a highly charged FG-Nup segment. Finally, the experimental dimensions of poly-Proline segments [37], poly-Glutamine [38], and poly-Glycine chains were used to fine-tune the hydrophobicity scales of Proline, Glutamine and Glycine residues, respectively.

With the Stokes radii of two FG-Nup segments used for parametrization, the model predicts the Stokes radii of the rest of the FG-Nup segments within 20% error with respect to the reported in-vivo values (see Fig. 1). In addition, the model captures the experimental brush height \( h = 14.1 \) nm) of end-grafted Nup62 segments with acceptable accuracy \( h = 13.0 \) nm.

2.2. Percolation analysis

When a sol–gel transition occurs, the system transforms from a viscous fluid into a viscoelastic material [40]. For a polypeptide solution this happens when the concentration exceeds a critical value \( c_{\text{crit}} \). At this stage a large interconnected cluster is formed in the system which forms the gel network. Such a sol–gel transition can be quantified using percolation theory [41].

We have used a finite-size scaling method to determine the critical concentration for an infinite system. This method relies on the percolation probability \( P \), defined as the probability of forming an infinite cluster in the system. The percolation probability versus concentration curves \( P(c) \) for systems with different sizes are obtained and the intersection of these curves has been shown to determine \( c_{\text{crit}} \) [42–44]. The percolation probabilities for different concentrations can be fitted to the function

\[
P(c) = 1 - \left( 1 + \exp \left( \frac{c - b}{a} \right) \right)^{-1},
\]

where \( a \) and \( b \) are size-dependent fitting constants [45,46]. The fitting curves are used to quantify the intersection point of the \( P(c) \) curves (as shown, for instance, in Fig. 3).

2.3. Simulation setup

Simulations are performed using the 1BPA molecular dynamics method described in Section 2.1. The FG-Nups are placed in a cubic box of linear size \( L \) with periodic boundary conditions and Langevin simulations are performed at 300 K (see Fig. 2A). The concentration of the FG-Nup solutions is varied by changing the number of FG-Nups, \( n \), while keeping the box size \( L \) constant.

In order to obtain the percolation probability \( P \), first the connectivity of the FG-Nup network for every simulation frame is analyzed. If at least one residue of two different FG-Nups \( i \) and \( j \) comes closer than a distance \( d_{ij} = 0.7 \) nm, those Nups are considered to be in contact (see Fig. 2B). The choice of 0.7 nm has been made based on the equilibrium distance for hydrophobic interactions \( \sigma = 0.6 \) nm per 0.1 nm to account for thermal
fluctuations around the equilibrium point. A connectivity matrix $C$ is then constructed by comparison of all FG-Nups in the system

$$
C_{ij} = \begin{cases} 
0 & d_{ij} > 0.7 \text{ nm} \\
1 & d_{ij} \leq 0.7 \text{ nm}
\end{cases}
$$

Once the connectivity matrix is constructed, the largest cluster of the system is identified and the maximum dimension of the percolating cluster is calculated in the $x$, $y$ and $z$ directions. If the maximum dimensions are equal or greater than $L$, the system is considered to be percolated ($P = 1$) and otherwise the percolation probability $P$ is set to 0. Ultimately, $P$ is calculated by averaging over all the percolation probabilities calculated for the different simulation frames.

The FG-Nups systems are first energy minimized using a steepest descent method and then simulated for at least $8 \times 10^6$ steps and the first $10^6$ steps are not considered for the percolation analysis. The percolation probability $P$ is calculated by extracting the coordinates of the FG-Nups every $10^4$ steps. A convergence study on the number of simulation frames required to calculate the percolation probability is shown in Fig. A of the Appendix which shows that a good accuracy for $P$ can be reached by using 750 simulation frames.

3. Results

3.1. Network formation properties of FG-Nups

First, the gel formation of a Nsp1p (nsp1 AA 1-606) solution is investigated. The average percolation probability versus the Nsp1p concentration is calculated and plotted in Fig. 3. Given the large computation times, we use three different box sizes, confirming a unique intersection point. As expected, larger clusters start to form in the system when the concentration of the FG-Nups in the solution increases. Our results predict a concentration of $c_{\text{crit}} = 27$ mg/mL for the Nsp1p system.

3.2. The effect of mutations

Next, the effect of mutations of the amino acid sequence of Nsp1p on the critical concentration is investigated. First, all hydrophobic F residues in the amino acid sequence of Nsp1p are replaced by the less hydrophobic S residues. The percolation analysis shows that the critical concentration increases to $53$ mg/mL for F to S mutated Nsp1p solutions (see Table 1 and Fig. 4A for the $P$ versus $c$ curves). Second, we tested the hydrophobic-to-hydrophobic F to Y mutation of Nsp1p. This mutation has a much lower effect on the gelation properties of Nsp1p ($c_{\text{crit}} = 38$ mg/mL, see Table 1 and Fig. 4B). This supports the premise that the cluster formation of the FG-Nups is mainly driven by hydrophobic interactions.

3.3. Charge and hydrophobicity effect

From the results of the mutated Nsp1p it can be hypothesized that cluster formation in FG-Nup solutions is promoted by the attractive interaction between hydrophobic amino acids, whereas the repulsive interaction between hydrophilic charged amino acids opposes this effect.

We have investigated this hypothesis by analyzing several FG-Nups with different charge and hydrophobic contents (i.e., Nsp1n, Nup60, Nup100 and Nup116s, see Fig. 5). The hydrophobicity of the FG-Nups is measured by the sum of the hydrophobic strengths $\varepsilon_i$ of the individual amino acids in the sequence of the FG-Nup, $H = \sum_{i=1}^{N} \varepsilon_i$, where $N$ is the total number of amino acids of the FG-Nup, with the $\varepsilon_i$ values of the different amino acids given in [24]. Additionally, the charge content of the FG-Nups, $C$, has been measured by counting the number of charged amino acids in the sequence of the FG-Nups. Consequently, $C$ and $H$ are both dimensionless positive numbers (i.e., integer and real, respectively).

It has been shown that the critical concentration of polymer solutions depends on the length of the polymers [44,46]. In order to exclude this effect from the results, the charge to hydrophobicity ratio ($C/H$) for each FG-Nup is normalized by the scaling relation ($R_g \propto N^{0.589}$) of the gyration radius $R_g$ obtained for disordered proteins in the absence of any hydrophobic and electrostatic interaction [23]. The critical concentrations $c_{\text{crit}}$ for several FG-Nup
systems is plotted against the normalized charge to hydrophobicity ratio in Fig. 6 (see also Table 1). The results suggest that as the charge to hydrophobicity ratio of the Nups increases, the sol–gel transition occurs at higher concentrations.

### 4. Discussion and conclusion

The gel formation properties of FG-Nups are investigated by means of a percolation analysis of the conformations of FG-Nup solutions, using a recently developed one-bead-per-amino acid coarse-grained model. The results indicate that a sol to gel transition occurs at $c_{\text{crit}} = 27 \text{ mg/mL}$ for Nsp1p solutions, which is more than a factor of two larger than the experimental critical concentration of 10 mg/mL [12]. This discrepancy might be partly due to the fact that the definition of gel-formation is different: through percolation analysis in the modeling and through visual

### Table 1
The FG-Nup segments used for the percolation analysis with their length, charge to hydrophobicity ratio $C/H$ and critical concentration.

<table>
<thead>
<tr>
<th>FG-Nup</th>
<th>Length</th>
<th>$C/H$</th>
<th>$c_{\text{crit}}$ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nsp1p</td>
<td>606 (AA 1-606)</td>
<td>0.460</td>
<td>27</td>
</tr>
<tr>
<td>Nsp1p F→S</td>
<td>606 (AA 1-606)</td>
<td>0.524</td>
<td>53</td>
</tr>
<tr>
<td>Nsp1p F→Y</td>
<td>606 (AA 1-606)</td>
<td>0.484</td>
<td>38</td>
</tr>
<tr>
<td>Nsp1n</td>
<td>172 (AA 1-172)</td>
<td>0.035</td>
<td>40</td>
</tr>
<tr>
<td>Nup60</td>
<td>151 (AA 389-539)</td>
<td>0.508</td>
<td>58</td>
</tr>
<tr>
<td>Nup108s</td>
<td>190 (AA 611-800)</td>
<td>0.539</td>
<td>75</td>
</tr>
<tr>
<td>Nup116s</td>
<td>195 (AA 765-960)</td>
<td>0.754</td>
<td>90</td>
</tr>
</tbody>
</table>
Nupsolutions. In addition, we show that, with increasing charge to hydrophobic interactions, sol–gel transition in FG-Nups by using a percolation analysis. Our results confirm a mixed phase inside the core region.

It has been suggested that FG-Nup hydrogels are initially formed through hydrophobic interactions which result into the formation of high molecular weight clusters and are subsequently stabilized by intermolecular β-sheets [47]. The hydrophobic interactions were mentioned to be the main driving force for gel formation of FG-Nups rather than hydrogen bonds, because FG-Nups with mutated hydrophobic residues failed to form a hydrogel [17,47]. Our results on different FG-Nup systems show that the critical concentration is related to both the charge and the hydrophobic content of the FG-Nups. The hydrophobic amino acids contribute to cluster formation, while interactions between hydrophilic, charged amino acids tend to be repulsive and hence prevent FG-Nup clusters to form.

Care should be taken in making a direct comparison between the current results and gel formation inside the NPC. Inside the NPC, the FG-Nups are anchored to the scaffold, while in our percolation analysis the FG-Nups are free in solution. The anchoring puts constraints on the FG-Nups to cluster together compared to Nups that are free in solution, which is expected to enhance the critical density of the sol–gel transition inside the NPC. In addition, the solutions analyzed here consist of one type of FG-Nup with equal length, while inside the NPC different types of FG-Nups are present, with rather different amino acid sequences and varying lengths. Despite these differences, our results can provide qualitative insights on the propensity to gel formation of the FG-Nups inside the NPC. As was shown in [24], the FG-Nups form a non-uniform distribution with a low density region at the center of the pore, surrounded by a high density donut-like region that is rich in FG-repeats. Comparison of the densities of the donut-like region (in the range from 200–300 mg/mL for viable yeast strains and 300 mg/mL for wildtype NPCs) with the critical densities of the solutions studied here (in the range from 27 to 90 mg/mL, see Fig. 6), suggests that the high density donut-like region is prone to form a hydrogel inside the pore. However, it is interesting to note that the FG-Nup densities in the center of the NPC that control passive transport (from 40 to 110 mg/mL [48]), overlap with the range of critical densities observed here, thus suggesting at most a mixed phase inside the core region.

In conclusion, we have investigated the gel formation properties of FG-Nups by using a percolation analysis. Our results confirm that hydrophobic interactions can lead to a sol–gel transition in FG-Nup solutions. In addition, we show that with increasing charge to hydrophobicity ratio, the critical concentration for gel formation increases. Comparison of the computed critical densities with previous calculations on the yeast NPC suggests a high probability for gel-formation to occur in a donut-shaped region that is rich in FG-repeats.

Acknowledgments

The authors acknowledge the Zernike Institute for Advanced Materials for financial support and NWO and the Dutch National Supercomputer Facilities (SURFsara) for granting computational time.

Appendix

Fig. A contains a convergence study on the number of simulation frames used to calculate the average percolation probability for different numbers of FG-Nups. The results show that convergence has been reached for 750 simulation frames.

References


