A Spring-Loaded Mechanism Governs the Clamp-Like Dynamics of the Skp Chaperone

Daniel A. Holdbrook¹, Björn M. Burmann², Roland G. Huber¹, Maxim V. Petoukhov⁴, Dmitri I. Svergun⁴, Sebastian Hiller²,* Peter J. Bond¹,³,*

¹Bioinformatics Institute (A*STAR), 30 Biopolis Str, #07-01 Matrix, 138671 Singapore
²Biozentrum, University of Basel, Klingelbergstrasse 70, 4056 Basel, Switzerland
³Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543 Singapore
⁴European Molecular Biology Laboratory Hamburg, c/o DESY, Notkestrasse 85, 22607 Hamburg, Germany

*Correspondence

Dr. Peter J. Bond
(peterjb@bii.a-star.edu.sg)

Prof. Dr. Sebastian Hiller
(sebastian.hiller@unibas.ch)
Abstract

The trimeric periplasmic holdase chaperone Skp binds and stabilizes unfolded outer membrane proteins (OMPs) as part of bacterial OMP biogenesis. Skp binds client proteins in its central cavity, thereby reducing its backbone dynamics, but it is unknown which molecular mechanisms govern Skp dynamics and how the chaperone adapts to differently sized clients. Here, we employ a combination of microsecond-timescale molecular dynamics (MD) simulation, small-angle X-ray scattering (SAXS) and NMR spectroscopy to reveal that Skp is remarkably flexible, and features a molecular spring-loaded mechanism in its “tentacle” arms that enables switching between two distinct conformations on sub-millisecond timescales. The conformational switch is executed around a conserved pivot element within the coiled coil structures of the tentacles, allowing expansion of the cavity and thus accommodation of differently sized clients. The spring-loaded mechanism shows how a chaperone can efficiently modulate its structure and function in an ATP-independent manner.
The outer membrane (OM) of Gram-negative bacteria functions as a general selectivity barrier. It provides the cell with protection from potentially harmful agents in the environment, while allowing useful molecules to enter. In order to fulfill this function, transmembrane β-barrel OMPs act as selective and non-selective porins. Their absence severely inhibits nutrient collection and therefore growth. OMPs are synthesized in the cytosol of the bacterial cell, and as such they follow a complex biogenesis pathway [1]. Unfolded OMPs traverse both the hydrophobic inner membrane and the hydrophilic periplasm before they are folded and integrated into the OM [2–4]. Due to their architecture as transmembrane proteins, OMPs are prone to misfolding and aggregation in the aqueous, but crowded periplasmic compartment. Undesirable intra- and inter-molecular interactions in the periplasm are typically prevented by a network of periplasmic chaperones including SurA, DegP and Skp [5,6]. These aid in the proper transport, folding and insertion of OMPs into the OM. Skp binds to unfolded OMPs as they emerge from the Sec translocon at the inner membrane. The binding of Skp prevents premature folding of OMPs, and promotes their release from the Sec complex once translocation is complete. Skp holds the OMP in a folding competent state [7], prior to arrival and release at the OM [8].

Skp is a trimer with a “jellyfish”-like architecture [9,10]. A small β-sheet “head” domain is the major site of association between the monomers, while three long, hairpin-shaped α-helical “tentacles” or “arms” define the outer boundaries of a central cavity, characteristic of a clamp-like binding site typically observed in chaperones [11]. Unlike the structure of prefoldin [12], a eukaryotic chaperone with related architecture, in the structures of Skp the α-helical extensions may
interact with one another at the tips. Each tentacle is composed of a short α-helix ($\alpha_1$) that leads from the β-sheet head into two extended antiparallel α-helices ($\alpha_2$ and $\alpha_3$). The OMP substrates of Skp are diverse in shape and size. The smallest of these OMPs, such as OmpA, have an 8-stranded β-barrel with diameter of 3.0 nm, whereas the largest, LptD, is 24-stranded [13]. Studies of Skp–OmpX conformation and dynamics have shown how Skp can bind 8-stranded OmpX and tOmpA [14], but it is yet unclear how Skp is able to adapt sufficiently to accommodate different larger substrates. NMR studies have identified a hinge region around Val42 / Phe50 between α-helix 1 and 2 that may allow the tips of the tentacles to move away from one another, thereby expanding the size of the central cavity to allow larger substrates to enter [14]. Such substantial flexibility in the α-helical regions of Skp is also consistent with absence of electron density for parts of the tentacles in both of the X-ray structures, and with structural disparities observed towards the tips when comparing the resolved subunits.

Here, we utilize extended, microsecond-timescale, atomic-resolution MD simulations of substrate-free (apo) trimeric Skp to explore its conformational landscape, and compare the results with data from small-angle X-ray scattering (SAXS) and amide–amide distances obtained from NMR spectroscopy. Unbiased simulation sampling is used to demonstrate that the Skp trimer is extremely flexible, revealing the full range of possible motion of the tentacles. Based on these observations, we propose hypothetical closed- and open-state models and identify a switching mechanism that leads to large variations in the volume of the Skp central cavity via the exchange of a helical kink between helix $\alpha_2$ and $\alpha_3$. 
This allows us to define the limits of Skp’s conformational mobility, which explains its capacity for binding substrates of variable size. Subsequent fitting of simulated conformational ensembles to data from SAXS measurements supports the notion that apo Skp exists in a dynamic equilibrium between open and closed states in solution. Finally, comparison of the simulations with NOESY experimental data confirms that apo conformations observed spectroscopically are well represented in the simulation trajectories, and that they are distinct from the substrate-bound form of Skp.

Results

Opening of the Skp Cavity via Separation of the Tentacles

The X-ray structure of Skp (PDBID: 1SG2) represents a “closed” state of the protein, with a tip-to-tip distance of < 0.78 nm measured between the Cα carbons of Ala76 [9]. Complemented by a partially modeled third subunit, the 1SG2 structure served as the initial seed for 15 independent MD simulation replicas of at least 100 ns in length. These simulations served to search for possible open states. Starting from the “closed” state, dissociation of the helical tips, as identified by a tip-to-tip distance > 1.4 nm, was observed in three of the 15 simulations, in a manner that was insensitive to initial conditions [8]. These separations of the two subunits towards an “open” state occurred in all three cases after >80 ns of simulation.

Following the observation that the tentacle-like arms were able to separate from one another on the nanosecond time scale, the dynamics of Skp were investigated further in two significantly extended simulations, of 1 μs in length.
each, beginning in the “open” conformation. Additionally, we measured SAXS profiles of a sample of highly pure apo Skp at a synchrotron beamline. During the two 1 µs MD simulations, the tips were observed to spontaneously re-associate and disassociate at three different time points. The re-association of the tips was apparent in the measured radius of gyration ($R_{\text{gyr}}$) of Skp (Figure 1A), which dropped below the $R_{\text{gyr}}$ of the X-ray structure (3.0 nm) at ~250 ns in one simulation and at ~80 ns and ~850 ns in the other. The average $R_{\text{gyr}}$ of 3.28 nm observed in the 1 µs MD simulations (Figure 1B) agrees well with experimentally measured $R_{\text{gyr}}$ of 3.3 nm for the apo-Skp trimer in solution, determined previously by neutron scattering [15]. It thus also agrees well with the $R_{\text{gyr}}$ of 3.6 nm determined by SAXS, considering that the scattering of the hydration shell likely adds ~0.6 nm to the triaxial dimensions of the protein in SAXS measurements [16,17].

The experimental determinations of $R_{\text{gyr}}$ by SAXS and neutron scattering are ensemble averages and thus mask individual, short-lived conformational states. Indeed, the structures determined by MD simulation feature a wide range of individual $R_{\text{gyr}}$ values, ranging between 2.9 and 3.7 nm (Fig. 1A). Thereby, the larger values of $R_{\text{gyr}}$ corresponded to conformations of Skp where the helical arms are projected away from the trimeric axis of symmetry, in some cases dramatically exposing the large central cavity (Figure 1C–I). Previous simulations that constrained Skp to explore a particular $R_{\text{gyr}}$ hinted at such expansion of the central cavity [15]. On the other hand, the smaller values of the $R_{\text{gyr}}$ correspond to conformational states of Skp where the tips of all three helical arms are re-associated with one another (Figure 1C–II). In the distribution of tip-
to-tip distances, these closed conformations populate a distinct state at 2 nm,
well separated from the conformational ensemble continuum of the partially and
fully open state (Figure 1D). When the Skp arms are separated, the tip-to-tip
distance is typically between 3 to 7 nm (Figure 1D), with the distribution of
distances peaking at 6 nm. The maximal distance between the tips of two
subunits encountered in free simulation was 7.2 nm. Overall, these data thus
reveal an extraordinary degree of flexibility of the Skp arms in the apo state, in
agreement with solution NMR dynamics measurements [14]. These motions
average out on the sub-ms time scale, resulting in NMR-spectroscopically
equivalent resonances for the entire trimer.

“Spring-Loaded” Dynamics Characterize the Helical Arms

It has been suggested that a pivot element exists around a highly conserved
phenylalanine residue (Phe50) in helix α2, allowing for a conformational change
and increasing the volume of the central cavity [8]. In agreement with this
hypothesis, multiple observations of a spontaneous conformational change and
rotation around Phe50 were observed during simulation. This change involved
the exchange of a helical kink from initially bent helix α2 (Figure 2A - I) to
initially straight helix α3 (Figure 2A - II), and resulted in lateral projection of the
tip of the helical arm from the three-fold axis of symmetry. An example of this
transition is illustrated in Supplementary Movie 1. The kink in helix α3 was most
often accommodated from Ala100 to Asp105. In total, the conformational
transition at the helical kink was observed twelve times during the simulation
trajectories (Figures S1), but never occurred simultaneously in two or three
subunits. In all observed cases, the exchange of the kink from helix α2 to α3
exhibited all-or-nothing mechanics, and had a maximum lifetime of ~60 ns (Figure S1). The kink exchange had a simultaneous effect on the physical dimensions of the Skp trimer, with a directly coupled increase in the $R_{gyr}$ from 0.15 to 0.30 nm. This opened the cavity and may thus well play a role in substrate binding.

**Defining the Maximal Capacity of the Skp Cavity in the Open State**

To isolate the biologically relevant, dominant concerted motions of Skp, we removed the high-frequency background noise by performing principal component analysis (PCA) on the combined (2 x 1 μs) MD trajectories of the entire, trimeric Skp assembly (Figure 3). The exchange of the helical kink between helix $\alpha_2$ and $\alpha_3$ did not appear in the lowest frequency modes (Figure 3A, Figure S2), indicating that this large, switchable inter-subunit motion of an arm from Skp occurs independently of the other local subunit dynamics. This finding implies that allosteric communication between Skp subunits is absent. In light of this, we therefore investigated the internal arm domain motions by performing PCA on an “artificial” 6 μs trajectory composed of the individual subunit trajectories from each simulation (i.e. 3 subunits x 2 simulations x 1 μs each). Principal component 1 (PC1), the dominant motion of the Skp subunit (Figure 3Bi), accounted for over half of the total structural variance. This component involved outward projection of the tips of the tentacles, away from the three-fold symmetry axis of Skp, with a concurrent exchange of the helical kink from helix $\alpha_2$ to $\alpha_3$, as described above, whilst maintaining a stable head domain conformation (Figure S3). The spring-loaded movement is thus a key element of Skp tentacle dynamics.
In order to estimate the possible range in size of the central cavity, two structural states, termed “extreme closed” and “extreme open”, were constructed by applying $C_3$ symmetry to the two extreme structures of PC1 (Figure 4A). In both cases, a least-squares-fit to the β-sheet head domain of the X-ray structure was performed in order to position each subunit in the extreme models. The transition between these states is illustrated in Supplementary Movie 2. While the tips of the helices were observed to extend up to 7.2 nm from one another during free simulation, the symmetric “extreme open” model features a distance between tips of 12 nm. The dimensions of the resulting cavity enclosed by Skp were estimated by expanding a virtual sphere at intervals along the axis of symmetry. In the “extreme closed” model, a maximum radius of 1.75 nm for the expanding sphere was achieved at a depth of ~2.0 nm beneath the head domain (Figure 4B). This position corresponds precisely to the height of the kink in helix α2. From this point onwards, the radius tapers, decreasing towards the tips of the helical arms. In the “extreme open” model, the upper part of the central cavity, immediately below the head domain, has similar dimensions to the closed model. However, the radius of the sphere continues to increase linearly, reaching a maximum radius of 3.2 nm at a 4.0 nm distance from the head domain. Using NMR-based distances measurements, it was previously shown that when bound to Skp, 8-stranded OmpX, one of the smallest possible substrates, adopts to a first order approximation a spherical ensemble of conformers with a radius of 2.1 nm. This fluid globule state can already be nearly accommodated within the “extreme closed” state of apo Skp, whereas larger substrates might be accommodated in the Skp cavity by gradual opening of the arms towards the
“extreme open” state. Extrapolating under the assumption of equal mass density from unfolded OmpX to the large 22-stranded substrate FhuA, the latter polypeptide would adopt a spherical volume with 3.5 nm radius. This large substrate might even therefore be accommodated at the lower end of the cavity in the extreme open state; conceivably, an additional Skp trimer may also be recruited for binding [7].

SAXS Reveals a Dynamic Ensemble of Open and Closed States

For a description of the Skp apo state, the experimentally determined SAXS intensity was compared with theoretical calculations (Figure 5). Because the individual conformers of the simulation trajectories feature substantial variability in the conformations adopted, their calculated intensities vary (see Figure S4 for the range of theoretical scattering intensities and indicative structures), and no intensity computed from a single structure described the experimental SAXS data within experimental error. Likewise, the scattering computed from the X-ray crystal structure did not agree with the SAXS data, as evidenced by a discrepancy in the goodness of fit, with $\chi^2 = 5.9$. A better agreement with the experimental data was obtained by allowing for mixtures of individual conformers, i.e. linear combinations of the computed patterns. The ensemble optimization method (EOM) [18,19] uses a genetic algorithm to recombine models from a pool of structures, until an optimal fit to the experimental SAXS curve is obtained. In order to include as many maximally open states in the dataset, the pool of Skp structures from the simulation trajectories was enhanced with an equal number of hypothetical symmetrical structures, created by applying $C_3$ symmetry to a random selection of individual
subunit conformations. The combined pool contained structures that had $R_{\text{gyr}}$ values in the range 2.75 to 4.25 nm (Figure 5A, green curve). These structures generated a fit to the experimental curve with a $\chi^2 = 1.0$ (Figure 5B).

Representative structures obtained from the fit were similar to the “extreme open” and “extreme closed” models (Figure 5i, Figure 5ii). Thus, the SAXS experiments directly indicate that apo Skp in solution adopts a conformational ensemble, composed of variably opened and closed structures and their intermediate states.

While the fit to the experimental data was improved considerably by considering an ensemble of structures, there remained, however, a deviation at the low angles of the SAXS measurements, indicating a dearth of larger structures in the dataset. In order to increase the number of structures in the pool with a larger $R_{\text{gyr}}$, a new, independent random pool (Figure 5A, red curve) was generated using RANCH (RANdom CHain) within EOM [18,19], allowing the positions of the helical “tentacles” to vary with respect to the fixed “head” domain. EOM was again then employed to select an optimized set of models consistent with the data (Figure 5A, pink/cyan curve). This approach yielded a fit to the SAXS profile with a $\chi^2 = 1.5$ (Figure 5B), whereby the representative structures also included open and closed conformations (Figure 5iii, Figure 5iv, Figure 5v) further confirming the structural variability of Skp in solution. In this case, the selection frequency of structures indicated a bimodal distribution (Figure 5A, pink/cyan curve). The first peak corresponded to a $R_{\text{gyr}}$ of $\sim$3.2 nm, similar to that in the MD-generated pool (Figure 5A, green curve). The $R_{\text{gyr}}$ for the second peak ranged...
between 3.8 and 4.1 nm (Figure 5A), and was thus comparable to the R_{gyr} of 4.1 nm obtained for the "extreme open" model of Skp generated via PCA.

**NOESY Experiments Confirm Distinct Dynamics of Apo Skp**

The structural configuration of Skp in its apo state was investigated further by the calculation of a subset of inter-backbone amide hydrogen distances for each subunit configuration observed in the simulation trajectories (Figure 6A, Table S1). These distances were compared to distance assignments derived from NOESY spectroscopy for both apo-Skp and Skp bound to an OmpX substrate (Figure 6B, C). A narrow, unimodal distribution was obtained for the mean deviation of backbone distances of the tentacle domain across simulated Skp compared to the apo Skp NOEs, peaking at <0.8 Å. In contrast, the deviation from the OmpX-bound Skp NOE data exhibited a broader bimodal distribution, which only partially overlapped with the apo data, and extended further out, up to ~1.3 Å. Measurement of the equivalent distances for the X-ray crystal structure revealed that the three subunits of Skp deviate to varying degrees from both the apo- and substrate-bound NOE data. However, in all cases, the crystallographic deviations are increased compared to those for the most frequent conformations observed in the simulations. Thus, the simulated ensemble, characterized by a range of open and closed states, is best represented by ligand-free, apo Skp in solution. Its conformation is likely modulated by the presence of OmpX, possibly biasing the ensemble towards collapsed states that protect the substrate.
Discussion

Via a combination of simulation and experiment, we have shown here that ligand-free, apo Skp exists as a dynamic ensemble of multiple conformational states, with variable access to the central cavity. Interconversion between open and closed states is fast, with dramatic changes already on the microsecond timescale, as reflected in the observed frequencies of tip-to-tip separation, helical exchange between helix $\alpha_2$ and $\alpha_3$, and concomitant changes in protein $R_{gyr}$ and cavity volume. This finding is in full agreement with previous NMR measurements of Skp backbone dynamics, showing that complete conformational averaging is obtained in at most 1 ms [14]. The simulations thus reveal a novel mechanism for chaperone cavity expansion, involving spring-loaded dynamics of the tentacles, whereby a helical kink is exchanged between helices $\alpha_2$ and $\alpha_3$. This mechanism serves to allow the tips of each subunit to project away from the central axis of symmetry, whilst ensuring minimal disruption of the protein structural fold. It will be of great interest to assess whether architecturally similar (but evolutionarily distinct) eukaryotic chaperones such as Tim9/10 or prefoldin have related functional mechanisms [20,21].

The ability of Skp to adapt to a variety of differently sized substrates is key to its role in periplasmic OMP trafficking. This functional requirement is met by variability in the shape created by the helical tentacles. Thereby, the physical limit of the expansion of the Skp cavity is far greater in size than displayed in available X-ray crystallographic structures, and thus sufficient to accommodate Skp substrates above 8 strands. Skp substantially alters its conformation upon
substrate binding by reducing backbone dynamics and flexibility in the pivot region [14]. The substrate-bound state of Skp was previously found to be structurally distinct from the conformations explored by apo Skp, indicating a substrate-induced conformational change. The large-scale movements of the subunits relative to one another occur independently of both the exchange of the helical kink and of one another. Such loose, dynamic association of the Skp cavity with substrate ensures that the OMP is held in a folding competent state, without over-stabilizing non-native contacts of the OMP backbone [7]. The ability of Skp to expand its cavity in this way likely has functional impact both in the capture and release of diverse OMP substrates, and might also enable access to the central cavity for the OMP-inserting BAM complex [22–24].
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Experimental SAXS intensity of Skp and the structural models generated by EOM are deposited in SASBDB database (entries are being assigned).

Author Contributions

DAH and PJB performed the computational experiments; BMB, MVP, and DIS and SH performed the SAXS and NMR experiments; all authors analyzed the data; PJB, DAH, and SH wrote the paper with the contributions from BMB, RGH, MXP, and DIS; PJB and SH designed the study.

Competing financial interests. The authors declare no competing financial interests.

Materials & Correspondence. Correspondence and requests for materials may be directed to Prof. Dr. Sebastian Hiller (sebastian.hiller@unibas.ch) and Dr. Peter J. Bond (peterjb@bii.a-star.edu.sg).
Methods

Simulation System Configuration

A crystal structure of Skp (PDBID: 1SG2) [9] was used as an initial configuration.

The residue numbering convention used herein is based on UniProt entry sp|P0AEU7|21-161, where the first residue, Ala21, is the start of the mature protein chain. The helical tip residues of the third subunit, from Met60 to Ala95, are unresolved in the X-ray structure. The missing residues, therefore, were modeled with MODELLER [25], using the two resolved subunits as templates.

The initial tip-to-tip distance of the modeled subunit with regards to the other two subunits was 2.53 and 1.74 nm, respectively. 15 independent simulations, each of 100 ns in length, were initially performed of Skp, either in isolation, or in the presence of lipid A or lipid A + KDO bound at the putative LPS binding site on each subunit of the Skp homotrimer, as described in a previous study [8]. In accordance with the conclusions of this previous study, the simulations of lipid-bound Skp showed broadly similar dynamics to the lipid free simulations.

Snapshots of the “open state” of Skp defined from these preliminary shorter simulations were used to initialize 2 x 1 μs simulations. This was defined as the largest distance between the Cα of Ala76 residues (located at the tip of each subunit). The open Skp structures were solvated in a rhombic dodecahedron box, containing ~110,000 water molecules and a 0.15 M NaCl solution. Position restraints of 1,000 kJ mol⁻¹ nm⁻² were applied for 5 ns to the Cα atoms of the protein, prior to performing 1 μs production simulations.

Simulation Parameters and Analysis
Simulations were performed with the GROMACS simulation package [26,27], using the CHARMM22 force field parameter set, incorporating the CMAP potential corrections [28,29], as described previously [8]. All simulations were performed in the NPT ensemble at a temperature of 298 K and pressure of 1 atm. The temperature was controlled with the velocity-rescale thermostat [30], and pressure by the Parrinello-Rahman barostat using isotropic coupling [31,32]. A 2 fs timestep was used to integrate the equations of motion, and the LINCS algorithm was used to constrain all bond lengths [33]. A 1.2 nm cut-off was used for Lennard-Jones interactions, with the potential smoothly switched off between 1.0 and 1.2 nm. Electrostatic interactions were calculated using the Particle-Mesh-Ewald algorithm with a real-space cut-off of 1.2 nm [34]. VMD was used for visualization and creating images [35]. The VMD plugin, Bendix, was used to calculate the degree of bending in the helices and for the generation of images of kinked helices [36]. Other analyses were performed using GROMACS tools and in-house scripts that utilized the capabilities of MDAnalysis [37] and MODELLER [25].

SAXS Measurements and Primary Processing

SAXS measurements were performed at the P12 beamline of EMBL (DESY Hamburg) [38] covering the range of momentum transfer $0.01 < q < 0.44 \text{ Å}^{-1}$ ($q = 4\pi \sin(\theta)/\lambda$, where $\theta$ is the scattering angle and $\lambda = 1.2 \text{ Å}$ is the X-ray wavelength). Samples of Skp were prepared in the buffer containing 25 mM Hepes (pH 7.5), 150 mM NaCl, 1 mM DTT. Data was acquired in a range of protein concentrations from 5.8 to 0.6 mg/ml and analyzed using the ATSAS software package [39]. The primary data processing was performed using
The forward scattering $I(0)$ and the radii of gyration $R_g$ were evaluated using the Guinier approximation [41], assuming that at very small angles ($s < 1.3/R_g$), the intensity is represented as $I(s) = I(0) \exp(-sR_g^2/3)$. The maximum dimensions $D_{\text{max}}$ were computed using the indirect transform package GNOM [42], which also provides the distance distribution function $p(r)$.

**Comparison of MD-Derived Conformers to SAXS**

CRYSOL [43] was used to calculate the form factors for the structures derived from MD trajectories. Default values were used for the solvent density (0.334 e/Å³). All theoretical scattering curves derived from simulation structures were fitted to the experimental data.

**Flexibility Assessment by Ensemble Optimization Method**

Ensemble Optimization Method (EOM) [18,19] has been applied to characterize conformational variability of Skp in solution. The positions and orientations of the three helical arms (Asn20 – Ala115) were randomized with respect to the rest of the structure to generate a pool of 10,000 models. The genetic algorithm was applied to select from the pool an optimized ensemble that best fit the experimental SAXS data.

**Protein Expression, Purification and Isotope labeling**

Skp containing an N-terminal hexa-histidine tag and lacking its signal sequence, and OmpX obtained from inclusion bodies, were expressed and purified as described previously [14,44]. $[U^{-2}\text{H}, ^{15}\text{N}, ^{13}\text{C}]$-labeled Skp was obtained by growing the expression cells in M9-minimal media [45] supplemented with
\((^{15}\text{NH}_4)\text{Cl}, \text{D–}[^2\text{H}, ^{13}\text{C}]-\text{glucose and D}_2\text{O}. [U–^2\text{H}]-\text{labeled OmpX was obtained by the addition of D–[^2\text{H}]-\text{glucose and D}_2\text{O to M9 minimal medium. Isotopes were purchased from Sigma-Aldrich or Cambridge Isotope Labs.}

\textbf{NMR Spectroscopy}

NMR experiments of human Skp and Skp/OmpX were performed in NMR buffer containing 25 mM MES, 150 mM NaCl pH 6.5. The measurements were recorded at 304 K on a Bruker AscendII 700 MHz spectrometer equipped with a cryogenically cooled triple-resonance probe. The 3D \([^{1}\text{H},^{1}\text{H}]-\text{NOESY–}^{15}\text{N-TROSY}\) experiments [46–48] were recorded with a mixing time of 100 ms resulting in a total experiment time of 141.5 h for Skp in its apo form and for 112.5 h for Skp–OmpX. The interscan delay was set to 0.95 s. In the direct dimension, 1024 complex points were recorded in an acquisition time of 91 ms, multiplied with a 75°-shifted sine bell, zero-filled to 2048 points and Fourier transformed. In the nitrogen indirect dimension, 90 complex points were measured with a maximal evolution time of 40 ms, multiplied with a 75°-shifted sine bell, zero-filled to 256 points and Fourier transformed. In the proton indirect dimension, 150 complex points were measured with a maximal evolution time of 18 ms for Skp in its apo form, multiplied with a 75°-shifted sine bell, zero-filled to 512 points and Fourier transformed. For all spectra a polynomial baseline correction was applied in all dimensions. NMR data were processed using PROSA [49] and analyzed with CARA [50] and XEASY [51].

\textit{Comparison of Simulation and NMR observed distances}

A subset of amide hydrogen-hydrogen interactions was chosen for comparison of
simulation and NOESY data. The subset was chosen by comparing the distances of the extreme structures of PC1 and PC2 to the X-ray structure. Two amide hydrogens were selected to be in this subset if they were <6 Å in any structure, and if their distance differed by >1 Å compared to the X-ray structure. Only those residue pairs for which a clear crosspeak/diagonalpeak quotient for either apoSkp or Skp-OmpX could be measured were used in the final analysis. The distance, $d$, between two amide hydrogens, $i$ and $j$, was determined using the following Equation: $d_{ij} = c \cdot P_{ij}^{-1/6}$, where $P$ is the crosspeak/diagonalpeak quotient and, $c$ is a global constant obtained by minimizing the average distance between the NMR (both apoSkp and Skp/OmpX) and simulation data, with the additional constraint that no NOE distance could be >6 Å. In any given simulation frame, the deviation from experiment for an atom pair was measured as the absolute difference between the simulation distance and the distance estimated from the NOE signal. For amide hydrogen pairs without an NOE signal, the deviation was 0 if the simulated distance was >6 Å, and otherwise was calculated as the absolute difference from 6 Å.
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**Figure 1. Opening and closing of apo-Skp in solution.**

**A)** The radius of gyration timelines for Skp in two 1 µs trajectories are shown in red and blue. The lines indicated to the right of the graph represent: a*, the “extreme open” model (shown later in Figure 4); b*, the largest radius of gyration achieved during free simulation; c*, the SAXS estimate of the radius of gyration described herein (after removing the influence of the hydration shell), also equal to the estimate provided in [15]; and d*, the radius of gyration calculated for the crystal structure (PDB 1SG2). **B)** Distribution of the radii of gyration for Skp from both 1 µs trajectories. The pink line is the combined distribution. The other colored lines correspond to those described in A. **C)** The structures corresponding to I) the maximum and II) the minimum radius of gyration achieved in the simulations. **D)** The tip-to-tip distance between all pairs of subunits in the Skp trimer. The distribution is combined for both trajectories. The tip-to-tip distance was calculated as the distance between the Cα atoms of Ala76 in all subunit pairs. The line, d*, corresponds to the value from the crystal structure.
Figure 2. Dynamic exchange of a kink from helix α2 to α3 in the tentacle domain. A) Cylinder representation of the subunit in A, displaying the kink in helix α2 (state I), and helix α3 (state II). The other two subunits of Skp are displayed in grey for clarity. B) Estimated kink angle for helix α2 and α3 for one subunit over the course of a 1 µs simulation. The degree of bending is shown in the top left corner. An exchange of kink between helices may be clearly observed between the 400-500 ns time period.
Figure 3. Large-scale dominant motions of both the trimeric complex and individual subunits of Skp. A) The extreme conformations of the entire Skp trimer along principal components i) PC1 and ii) PC2. PCA was performed for the combined (2 x 1 μs) “open Skp” trajectories. When combined, PC1 to PC4 explained almost 90% of the total variance in the data. Between the two individual 1 μs trajectories, there was substantial similarity in these modes (covariance overlap, 0.68), indicating convergence in the subspace explored. The exchange of the helical kink between helix α2 and α3 (Figure 2) appeared in none of the first four low frequency modes, and was instead observed in the higher frequency modes, PC5 to PC9. B) The extreme conformations of the isolated subunit, along principal components i) PC1 and ii) PC2. PCA was performed for the combined trajectories of each individual protein subunit (6 x 1 μs), after least-squares fitting to the rigid head domain. PC1 represents a motion...
involving exchange of the helical kink and outward projection of the tips of the tentacle, and accounted for >50% of the total variance in the data. PC2 accounted for only 10% of the data, and involves a rotation of the helical tentacles relative to the β-sheet head in an orthogonal direction to PC1. In both A) and B), the arrows indicate the direction and magnitude of motion, and the percentage of the total variance explained by the principal component is shown beside each figure. Note the difference in distance scale in each figure.
Figure 4. Hypothetical model of the “extreme open” and “extreme closed” states of Skp. A) The “extreme open” and “extreme closed” models created by assuming the extreme states of principal component 1 (PC1) of the subunit dynamics are applied to each subunit of the Skp trimer simultaneously. B) The largest sphere radius, along the cavity axis, calculated for the “extreme open” and “extreme closed” states shown in A). The radius of gyration for the “extreme open” model is indicated in Figure 1A (a†).
Figure 5. Ensemble fitting to the experimental SAXS intensities. A) Relative frequency of structures with a particular $R_{\text{gyr}}$ in the MD (green) and RANCH (red) pools. The frequency of models selected from by EOM is shown for the RANCH pool (pink/cyan). B) The optimized ensemble of RANCH (red line) and MD (green) generated structures fitted to the experimental SAXS profile. Representative structures selected by EOM are shown for both the MD (i and ii) and RANCH (iii, iv and v) generated models.
Figure 6. NMR observed backbone amide-amide hydrogen distances for apo- and OmpX-bound Skp, compared to simulated apo Skp. A) The backbone structure of Skp, with residues in blue indicating those between which amide hydrogen distances were measured for comparison with NOE assignments. A hydrogen-hydrogen interaction was selected for analysis if the pair of atoms were < 6 Å from one another in the X-ray structure or the extreme PC1 and PC2 structures. A subset of these was selected from those interactions that differed by > 1 Å between the PC structures and the X-ray structure. Of these, only those residue pairs for which a clear crosspeak/diagonal peak quotient for either apo Skp or Skp/OmpX could be measured were used in the final analysis. B) Distribution across all simulation trajectories of mean deviations from amide hydrogen distances from NOESY spectra of apo- and OmpX-bound Skp. Deviation from experiment for an atom pair was measured as the absolute difference between the simulation distance and the distance estimated from the NOE signal. For amide hydrogen distances without an NOE signal, the deviation was 0 if the simulated distance was > 6 Å, and otherwise was calculated as the absolute difference from 6 Å. Deviations for each subunit of the X-ray structure are indicated with dashed lines. C) Assigned 2D strips from 3D $^{15}$N-edited-[$^{15}$N,$^1$H]-$^1$H-NOESY spectra taken at the indicated positions of 250 µM Skp in its apo form (cyan) and 250 µM Skp with bound OmpX (holo form, purple) in NMR buffer at 37 °C. Spectra were recorded with a NOESY mixing time of 100 ms. Broken lines indicate NOE connections. Broken circles indicate missing NOE cross-peaks in either the holo or the apo-state. ** denotes crosspeaks from adjacent planes.