Integrative Atomic-Level Structure Modeling of the General Import Pore Complex

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About 20% of the proteins of a eukaryotic cell are mitochondrial proteins. Since the mitochondrial DNA encodes only a very small number of proteins, the vast majority of mitochondrial proteins are synthesized from nuclear DNA as precursor proteins on cytosolic polysomes and must be imported into the organelle. Most mitochondrial proteins enter mitochondria via the outer-membrane translocator, the TOM (translocase of the outer mitochondrial membrane) complex. The translocase machinery, formed by seven integral outer membrane proteins in yeast, contains receptors for cleavable and non-cleavable preproteins and a general import pore (GIP) that mediates the translocation of both classes of preproteins into or across the outer membrane. The preproteins are initially recognized by the receptor Tom proteins, Tom20 or Tom70, and are then transferred to the GIP complex. This 400 kDa GIP complex consists of five proteins: Tom40, Tom22 and three small Tom proteins, Tom7, Tom6 and Tom5 [1]. In the last decade, information on the structural aspects of the TOM complex has been significantly accumulated [1], [2]. It has been established that the assembly of the mature multipore TOM complex depends on the presence of Tom22 [3].This central Tom receptor spans the outer membrane with a single α-helix and exposes soluble domains to the cytosol and the intermembrane space that both interact with incoming precursor proteins. In the absence of Tom22, Tom40 and small Tom proteins form small double-pore complexes [4], [5]. Recently, experiments using cross-linking between TOM40 and TOM22 have been performed [5], [6], which allowed conclusions about the relative positions of both proteins. Despite a significant progress in gaining structural insights into the TOM mashinery, high-resolution structures for most of TOM components and for full TOM-complex are absent. Here, computational structural biology tools, the recent experimental data on the structure of TOM complex components, the structure of the whole TOM complex obtained by electron microscopy and the data of cross-linking experiments were used together to obtain the integrative atomic-level structural model of the GIP complex.

The 3D-structures of yeast TOM40, TOM22, TOM5, TOM6, TOM7 were predicted using the I-TASSER protocol [7]. The modelling of the 3D-structures of the

protein-protein complexes was performed in a stepwise fashion with an initial rigid-body global search and subsequent steps of refinements to improve these initial predictions. To do this, four - stage computational molecular docking protocol PIPER [8]–ROSETTADOCK$_1$ [9] – HADDOCK [10] -ROSETTADOCK$_2$ (abbreviated as PRHR) was used. In several cases we modeled the interaction of a certain protein with a short peptide <30 residues. In these cases the program of flexible docking FLEXPEPDOCK [11] was applied. Clustering of structures and energy funnels were used to improve the ability of finding the correct structure of the complex. In the present work, the ranking by binding affinities among different complexes was based on the ROSETTADOCK$_2$ interface energy score (I_sc) instead on ROSETTADOCK binding score (RDBS) to bypass the problems of global minimization of pulled apart individual proteins. We categorized all protein-protein interactions into five classes (Very High-Strong transient (10^{-14}M<K_d<10^{-10}M; -16<I_sc<-12), High -Strong transient (10^{-10}M< K_d<10^{-8}M; -8.5> I_sc >-12), Medium - Strong transient (10^{-8}M< K_d<10^{-6}M; -6.5> I_sc >-8.5), Low - Strong transient (10^{-6}M< K_d<10^{-5}M; -4> I_sc >-5.5), and weak transient (K_d>10^{-5}M; I_sc > -4) in accordance with our data [12] on correlation between binding affinities and I_sc values. Such a categorization allows the approximate qualitative ranking between different complexes by affinities through their ranking by the ROSETTADOCK interface energy score (I_sc).

The strategy of the successive docking of TOM components was applied in accordance with the data from [13] on the sequence of the TOM complex assembly. At first we performed the modeling of interactions between the TOM40 and TOM22$_{TM}$ (the TOM22 transmembrane segment, residues 92-121) proteins. Among five structures provided by I-TASSER for each protein the highest-scored structure for TOM40 and an α-helical structure for TOM22$_{TM}$ were chosen and subjected to computational docking using the program PIPER [8]. Among 100 structures provided by PIPER one structure possessed the interaction type between TOM40 and TOM22$_{TM}$ with Lys94 and Glu120 of TOM22 being in close proximity to Arg310 and Asp350 of TOM40, in accordance with the recent cross-linking data of Shiota et al [5]. This structure was refined using the FlexPepDock program [11].

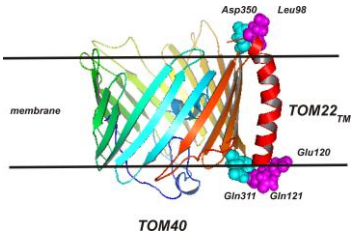

Fig. 1. Structural model of the TOM40/TOM22$_{TM}$ complex. The residues of TOM40 and TOM22$_{TM}$ forming intermolecular polar contacts are shown by cyan and magenta spheres, respectively

As a result, we obtained the structure (shown in Fig.1) with a good shape complementarity (BSA=2117 $Å^2$) and with three hydrogen bonds and one salt bridge between protein components. This resulted in a very low FlexPepDock I_{sc} value of -18.9. Because in a number of studies it has been shown that in the absence of TOM22 the predominant formation of the TOM40 dimer takes place [4], [5], we decided to address this issue by modeling the formation of TOM40 dimers and trimers using the PRHR docking strategy. Whereas no stable trimers were found (the highest-ranked trimer structure had the ROSETTADOCK$_2$ I_{sc} value of -4.0), a rather stable dimers were identified with the lowest ROSETTADOCK$_2$ I_{sc} value of -6.7. This low I_{sc} value was caused by a good shape complementarity (BSA=2286 $Å^2$) and formation of three hydrogen bonds between two monomers: Trp145TOM$_2$-Asn124TOM40$_1$, Trp145TOM40$_2$-Tyr143TOM40$_1$. Recently, the TOM complex architecture with three TOM40 and three TOM22$_{TM}$, each between two TOM40, has been proposed [5]. To address this issue in terms of atomic-level 3D-structures we performed the modeling of the interaction between two TOM40/TOM22$_{TM}$ dimers and then the interaction between the (TOM40$_1$/TOM22$_{TM,1}$/TOM40$_2$/TOM22$_{TM,2}$ tetramer and the TOM40$_3$/TOM22$_{TM,3}$ dimer. In the first case we obtained the tetramer structure with a fine shape complementarity between two dimeric components (BSA=4218.9 $Å^2$). This resulted in a low Isc value of -9.0. As to the small TOM proteins (TOM5, TOM6, TOM7), various combinations of their adding to the (TOM40/TM22$_{TM}$)$_3$ complex were explored. As a result, we obtained the atomic-level structure (shown in Fig.2) with three TOM40 proteins connected by the TOM22 proteins. This connection is stabilized by several small TOM proteins (four TOM7, two TOM5 and one TOM6).

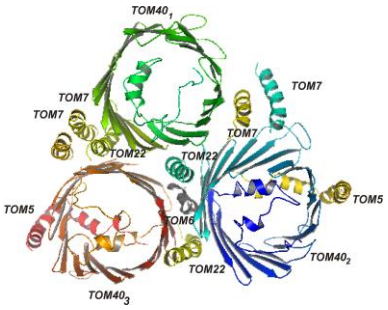

Fig. 2. Structural model of the TOM complex.

The results presented here suggest that the formation of the TOM complex occurs through an initial creation of basic structural blocks TOM40/TOM22 followed by the formation of the $(TOM40/TOM22)_3$ hexamers and addition to these complexes of small proteins TOM7, TOM6 and TOM5. Our simulations show that in the absence of TOM22, the formation of the TOM40 dimers is energetically favoured over the existence of individual monomers or the formation of higher order oligomers. Overall, our results provide atomic structure of the GIP complex, and explain in terms of atomic-level structures a number of experimental data

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