INTEGRATIVE MODEL OF THE YEAST TOM COMPLEX ATOMIC-LEVEL 3D-STRUCTURE

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Most mitochondrial proteins are imported into mitochondria from the cytosolic compartment by the outer membrane translocator complex (the TOM complex). The TOM complex consists of the channel-forming β-barrel protein Tom40 and six other subunits, each containing single α-helical transmembrane (TM) segments: the receptor protein TOM22, the regulatory small Tom proteins, Tom5, Tom6, and Tom7, and peripherally associated receptors, Tom20 and Tom70. In the last decade, information on the structural aspects of the TOM complex has been significantly accumulated [1]. It has been established that the assembly of the mature multipore TOM complex depends on the presence of Tom22 [2- 4]: in the absence of Tom22, Tom40 and small Tom proteins are found in small, double-pore complexes [4, 5]. Recently, experiments in yeast using cross-linking between TOM40 and TOM22 have been performed [3, 4] drawing conclusions about the relative positions of both proteins. Although there has been considerable 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 most 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 TOM complex. The 3D-structures of yeast TOM40, TOM22, TOM5, TOM6, TOM7 were predicted using the I-TASSER protocol [6]. 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 [7]–ROSETTADOCK$_1$ [8] – HADDOCK [9] -ROSETTADOCK$_2$ (PRHR) was used. 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 of on ROSETTADOCK binding score (RDBS). 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. At first we performed the modeling of interactions between the TOM40 and $TOM22_{TM}$ (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 for the use in subsequent simulations. Next, these two structures were subjected to computational docking using the program PIPER [8]. Among 100 structures provided by Piper (ClusPro server) we found one structure corresponding to 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 cross-linking data of Shiota et al [4]. Then this structure was refined using the FlexPepDock program [11]. As a result, we obtained the structure with a good shape complementarity (BSA=2117 $Å^2$) that formed three intermolecular hydrogen bonds and one salt bridge. This resulted in a very low FlexPepDock I_{sc} value (-18.9).

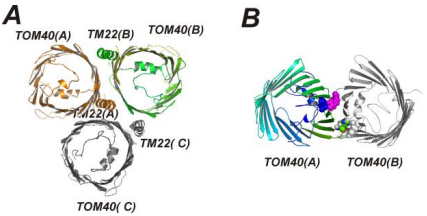

Fig 1. Structural models of the yeast $(TOM40/TM22)_3$ hexamer (A) and the $(TOM40)_2$ dimer.

Next we performed successively the modeling the 3D-structures of hexamers $(Tom40/TM22)_3$, TOM40 dimers ($(TOM40)_2$) and trimers ($(TOM40)_3$). Whereas no stable TOM40 trimers were observed (the highest-ranked trimer structure had the ROSETTADOCK(2) I_{sc} value of -4.0), a rather stable TOM40 dimers and (TOM40/TOM22) hexamers were identified with the lowest ROSETTADOCK(2) I_{sc} values of -6.7 and -8.1, respectively. These low I_{sc} values were caused by good shape complementarities (BSA=2286 $Å^2$ and 2970 $Å^2$, respectively) and three and seven hydrogen bonds, respectively, between interacting components. The structures of $(TOM40/TM22)_3$ and $(TOM40)_2$ are shown in Figure 1. The basic data of the simulations are summarized in Table 1.

Table 1. The ROSETTADOCK interface energy scores (I_sc), Buried Surface Area (BSA), number of intermolecular salt bridges (N_{sb}) and intermolecular hydrogen bonds (N_{HB}) for different highest- rank complexes

Protein complexes	Isc	BSA Å^2	N_{sb}	N_{nb}
TOM40 with $TOM22_{TM}$	-8.3	2117	1	3
TOM40 with TOM40	-6.7	2286	0	3
$(TOM40)_2$ with TOM40	-4.0	2323	0	1
(TOM40/TOM22) with (TOM40/TOM22)	-9.0	4217	0	1
$(TOM40/TOM22)_2$ with (TOM40/TOM22)	-8.1	2970	0	7

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