МОЛЕКУЛЯРНАЯ БИОФИЗИКА

ATOMIC-LEVEL MODELING OF THE HUMAN TOM COMPLEX 3D-STRUCTURE

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Mitochondria contain about 1000–1500 different proteins, most of which are synthesized as precursor proteins in the cytosol and imported into mitochondria via the outer-membrane translocator, 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 [1]. It has been established that the assembly of the mature multipore TOM complex depends on the presence of Tom22 [2-4]. 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 are found in small, double-pore complexes [5]. Despite a significant progress in structural characterization of the TOM complex, the molecular architecture of the complex still remains contradictory. Also the 3D atomic-level structural models of the complex are absent. Here, to obtain atomic-resolution structural model of the human TOM complex, we use 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 in yeast. 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 in yeast were used together to obtain the atomic-level structural model of the human TOM40/TOM22 complex. The modeling of human TOM40 and TOM22 3D-structures was performed using the I-TASSER protocol [6]. The prediction of the 3D-structures of the protein-protein complexes was performed in a stepwise fashion with an initial rigid-body global search and sub-

sequent steps to improve these initial predictions. To do this four - stage computational molecular docking protocol PIPER [7]–ROSETTADOCK$_1$ [8] – HADDOCK [9] -ROSETTADOCK$_2$ (abbreviated by 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 hTOM40 and hTOM22$_{TM}$ (residues 82-105). 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 to be used 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$_{TM}$ in yeast [4]. Then this structure was refined using the FlexPepDock program [11]. As a result, we obtained the structure with a good shape complementarity (BSA=1254 Å^2 for 24 TOM22$_{TM}$ residues). This resulted in a very low FlexPepDock I$_{sc}$ value (-17.3). 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 -2.0), a rather stable TOM40 dimers and (TOM40/TOM22) hexamers were identified with the lowest ROSETTADOCK(2) I$_{sc}$ values of -8.3 and -11.5, respectively. These low I$_{sc}$ values were caused by good shape complementarities (BSA=2392 Å^2 and 3235 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), Burried 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$_{nb}$
hTOM40 with hTOM22$_{TM}$	-8.3	1254 (24)	1	2
hTOM40 with hTOM40	-8.3	2392	0	2
h(TOM40)$_2$ with hTOM40	-2.3	1725	0	0
h(TOM40/TOM22) with h(TOM40/TOM22)	-10.9	3970	0	3
h(TOM40/TOM22)$_2$ with h(TOM40/TOM22)	-11.5	3235	0	10

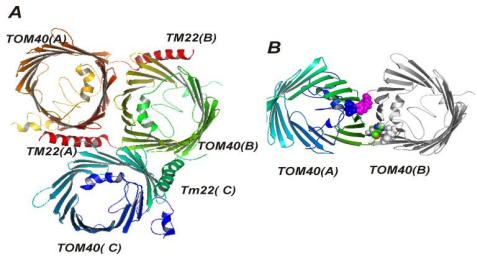

Fig 1. Structural models of the human hexamer $(TOM40/TM22)_3$ (A) and dimer $(TOM40)_2$ (B).

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