Molecular docking of mRNA 5' cap analogues containing aromatic substituents to human translation initiation factor eIF4E

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Background and Objectives

The eukaryotic translation initiation 4E factor (eIF4E) (Fig. 1) is a highly conserved small globular protein, which is responsible for recognition and selective binding of an mRNA 5' terminal regulatory structure called “cap”. The interaction of eIF4E with the 5’ cap is particularly important, since this is a rate-limiting step of initiation of protein biosynthesis and therefore plays a crucial role in cell development, growth and survival[1]. The elevated level of eIF4E leads to the efficient translation of oncoproteins and is closely related to the progression of cancer. Human eIF4E is thus thought to be a promissing target of anticancer therapy[2].

The newly synthesized cap analogue, **bz^{2}m^{7}GpppG**[6] proved to have significantly higher affinity to eIF4E than **(*p*-OCH$_3$-bz)^{2}m^{7}GpppG, bz^{7}GpppG**, **m$_2^{2,7}$GpppG** and **m^{7}GpppG** (Fig. 2)**.**

The eIF4E binding to the mRNA 5’ cap analogues is enthaply-driven and entropy-opposed (Fig. 3), which results mainly from interactions of the triphosphate chain with arginines and lysines, as well as from the cation-π sandwich stacking of m^{7}G ring in between W56 and W102.[5]

The goal of the studies was to obtain the putative 3D structures of of the eIF4E in the complex with novel cap analogues with aromatic substituents i.e, **bz^{2}m^{7}GpppG**[6]**, (*p*-OCH$_3$-bz)^{2}m^{7}GpppG** by molecular modeling.

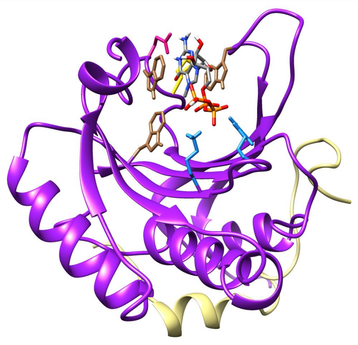

Figure 1. Crystal structure of human eIF4E in the ternary complex with the m^{7}GTP cap analogue and a eIF4G fragment (PDB id: *5t46*)[4]

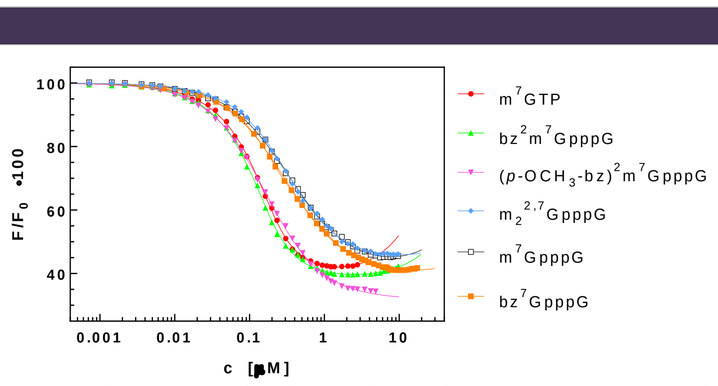

Figure 2. Fluorescence binding isotherms for eIF4E interactions with 5’ mRNA cap analogues at 294 K.

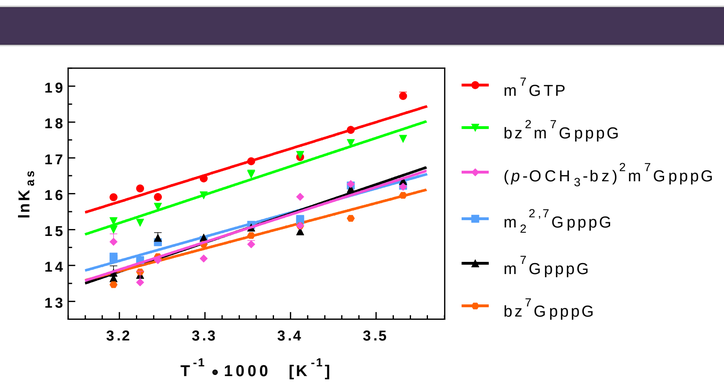

Figure 3. van’t Hoff plots for interactions of eIF4E with the mRNA 5’ cap analogues .

Molecular Docking

The protein models were prepared on the basis of the eIF4E structure deposited in PDB data bank (PDB id: *5t46*). Hydrogen atoms were added by UCSF Chimera including the ionizable residues which were protonated according to the physiological pH.

All mononucleotide 5’ mRNA cap analogues conformers were prepared on the basis of the m^{7}GpppA dinucleotide taken from the eIF4E complex (PDB id: *1wkw*) and the m^{7}GTP mononucleotide from the structure (PDB id: *5t46*) by using UCSF Chimera nad BioVia Discovery Studio programs. Hydrogen atoms were added to the conformers by UCSF Chimera program by taking into account protonation at the HN(1) position of 5’ mRNA cap analogues to get the cationic form.

Molecular docking were performed on the Rosetta Server[7]. The control compounds for the docking were m^{7}GTP, m$_2^{2,7}$GTP and bz^{7}GTP. In each docking, 200 ligand conformers were prepared by BCL tool with the starting coordinates from SDF files. The size of the grid was set as the maximum value of 30 Å. Two independent docking runs were performed for each 5’ mRNA cap analogue.

Acknowledgments
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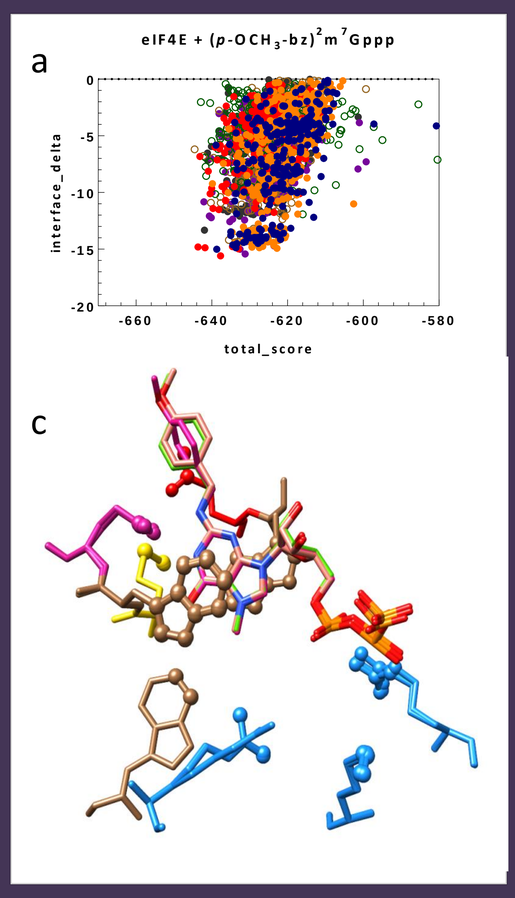

Figure 4. Correlation between change in the Gibbs free energy at 294 K determined from van't Hoff method based on equilibrium association constants derived from fluorescence titrations (Fig. 2) and the lowest values of the „interface delta” parameters obtained from the docking of the 5’ mRNA cap analogues: bz^{2}m^{7}Gppp-3, (*p*-OCH$_3$-bz)^{2}m^{7}Gppp-7, m^{7}GTP, bz^{7}Gppp-2, m$_2^{2,7}$Gppp-2. The „-3, -7, - 2” indicates the inner conformer.

Cap analogue	Δinterface delta	ΔG°$_{20°C}$ [kJ·mol^{-1}]
bz^{2}m^{7}Gppp-3	-15,51	-41 ± 6
(*p*-OCH$_3$-bz)^{2}m^7 Gppp-7	-15,11	-39 ± 7
m$_2^{2,7}$Gppp-2	-14,61	-38 ± 7
m^{7}GTP	-13,87	-38 ± 11
bz^{7}Gppp	-12,87	-37 ± 4

Results and discussion

1. Molecular docking (Fig. 3) yielded the 3D structures of the eIF4E complexes with **bz^{2}m^{7}Gppp** and **(*p*-OCH$_3$-bz)^{2}m^{7}Gppp**. Both structures are similar. The arrangement of the additional benzyl rings at the N^2 position is towards the solvent, in the vicinity of Q57, E103 and M101 residues.
2. The lowest „interface–delta” values obtained for the winners (Fig . 4) correlate with the the Gibbs free energy at 294 K calculated from van't Hoff method with R^2=0,875.

Conclusions and future directions

1. The aromatic benzyl rings at the N^2 position of **bz^{2}m^{7}Gppp** and **(*p*-OCH$_3$-bz)^{2}m^{7}Gppp** can enhance the dehydration effect by preventing the penetration of water molecules into the cap-binding pocket, thus leading to stabilization the protein-cap complex. This effect was not yet observed for additional benzyl groups.
2. The 5’ mRNA cap analogues containing the aromatic substituents at N^2 position are promissing compounds for the further design of eIF4E small molecule inhibitors.

References
[1] Siddiqui N. *et al*.; Biochem Soc Trans. 43(5):763-72 (2015).
[2] Prasad A. *et al*.; Clin Cancer Res. 1;19(1):85-95 (2013).
[3] Niedzwiecka A. *et al*.; Biochemistry; 43(42):13305-17 (2004).
[4] Gruener, S. *et.al.;* Mol Cell 64: 467-479 (2016).
[5] Niedzwiecka A. *et al.;* J. Mol. Biol., 319, 615-635 (2002).
[6] Piecyk K. *et al*.; Bioorganic & Medicinal Chemistry, 23 (15): 0968-0896 (2015).
[7] Deluca, S. *et al.;* PLoS ONE 10(7) (2015).