Multiple Targets Selection for Design of Inhibitors of Ebola Virus Infection

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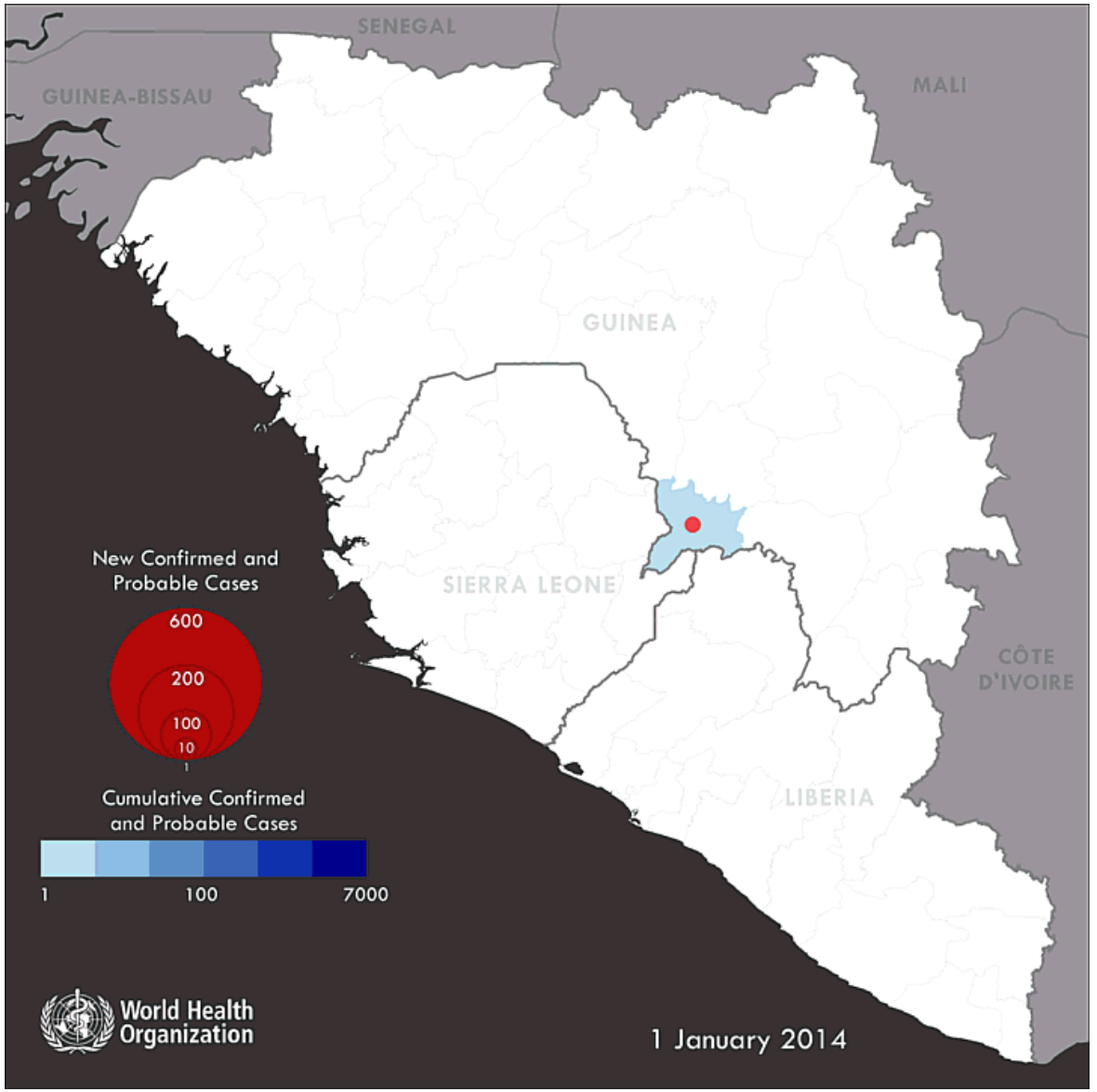
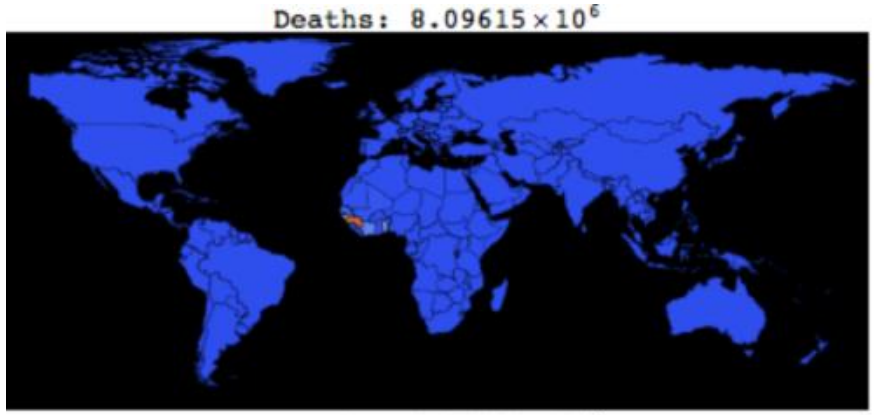
Pandemic Potential of Ebola

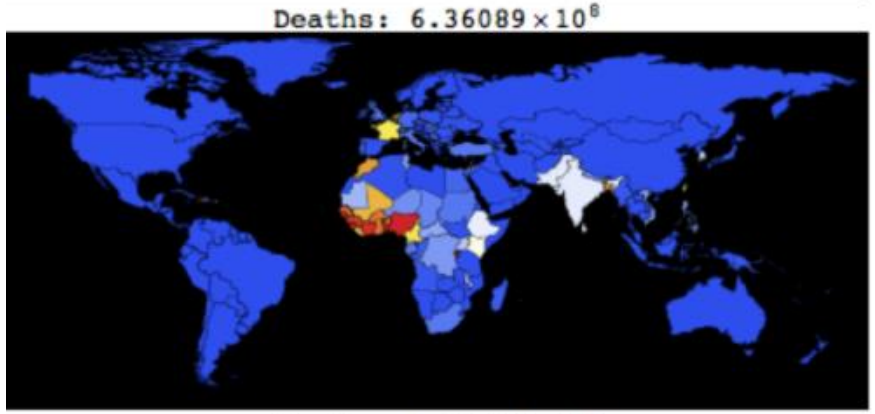


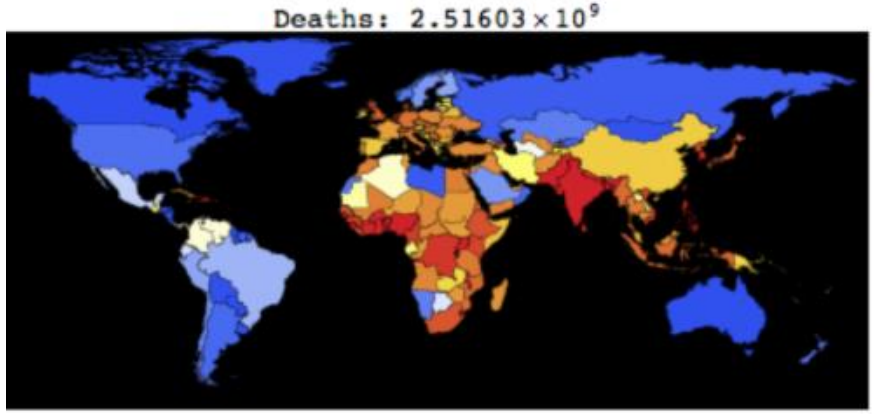
How to prevent pandemic threat of the Ebola

- **Early detection of EBOV and prompt action.**
- **Education of local population (change of burial habits ...)**
- **Re-Organization of health service**
- **Quarantine & Limitation of travels**
- **<u>Research of EBOV</u>**
  - **Epidemiologic studies**
  - **Genomics of EBOV**
  - **Structural Biology**
  - **Drug design**
  - **…..**

Pandemic Ebola – The worst scenario







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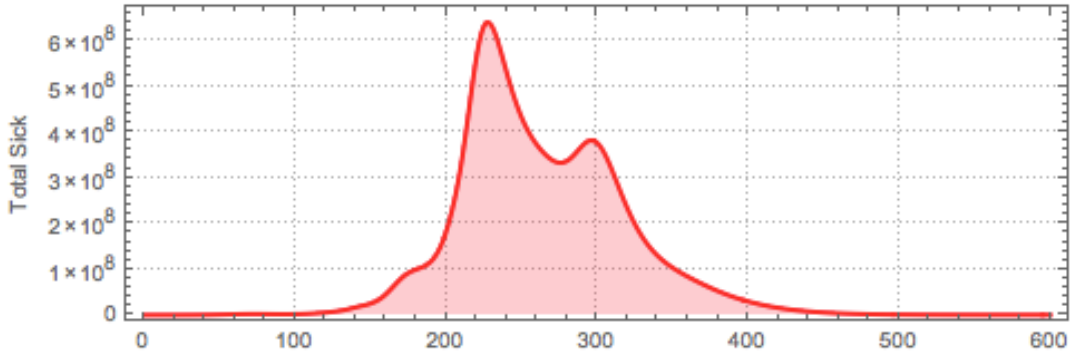

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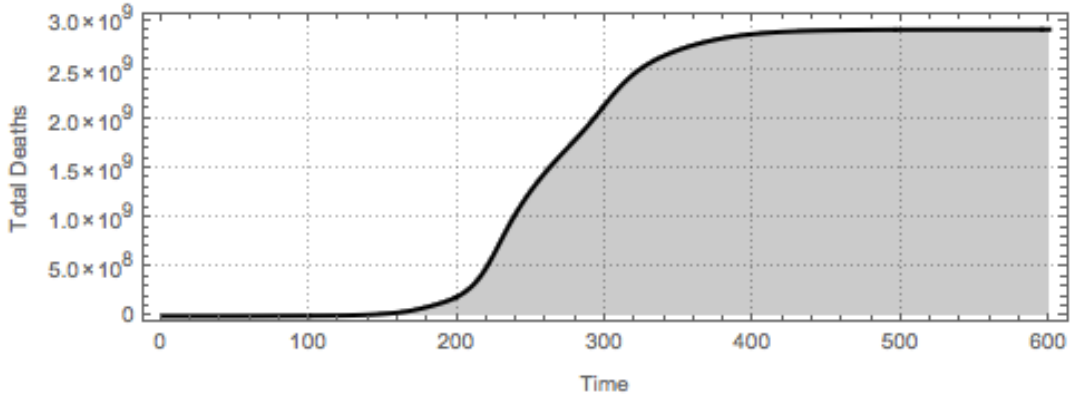
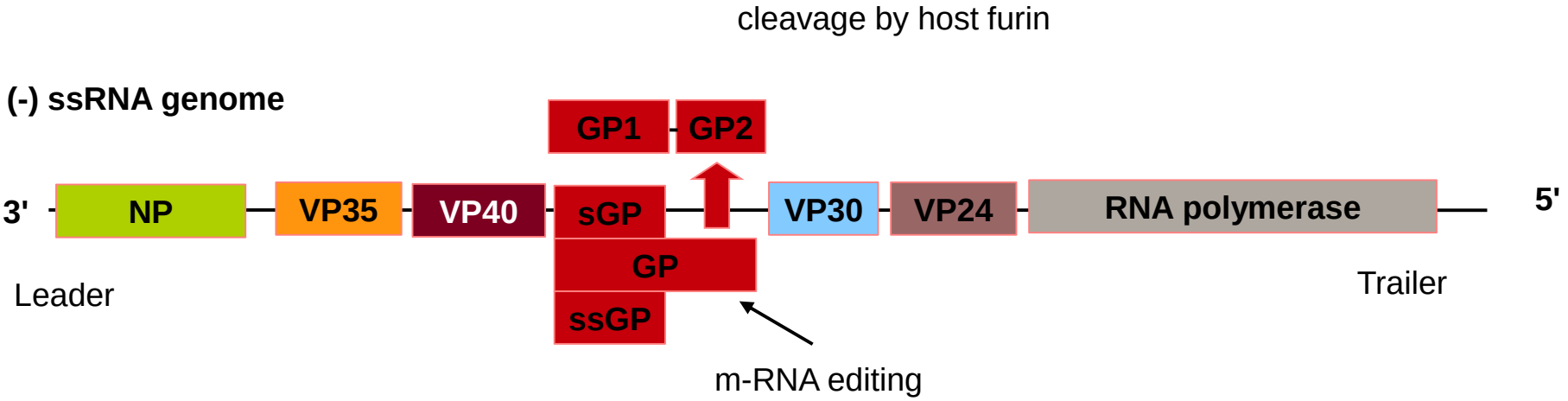
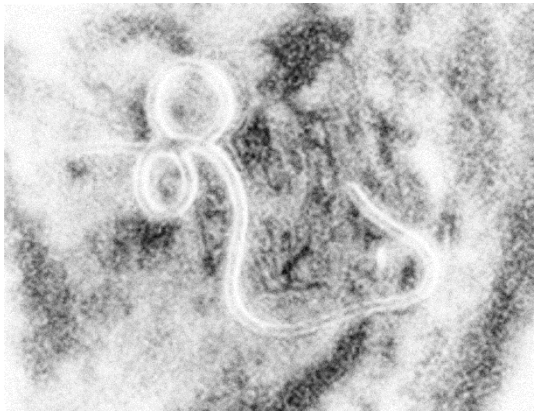


http://blog.wolfram.com/2014/11/04/modeling-a-pandemic-like-ebola-with-the-wolfram-language/

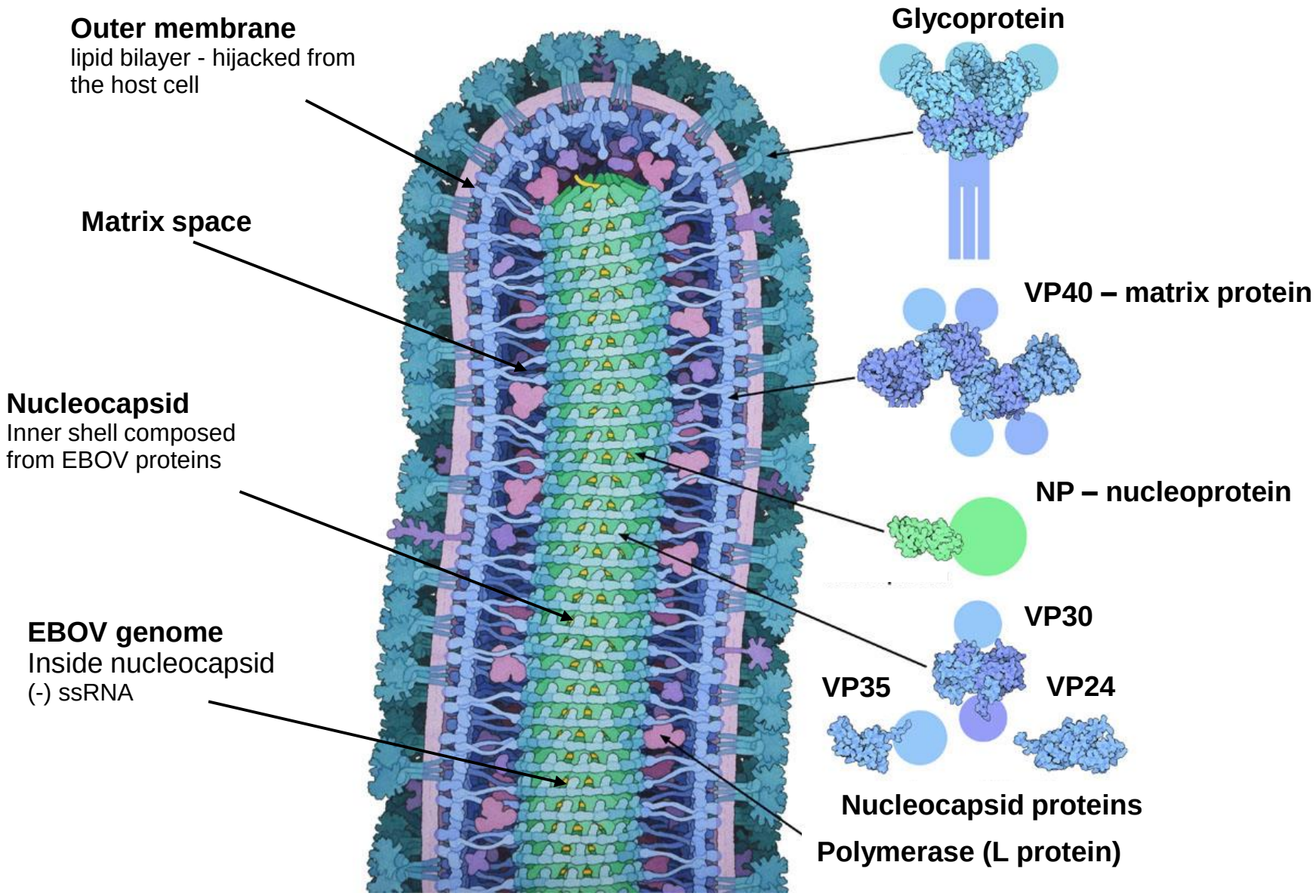
Simplicity of Ebola Virus





- filovirus family (*Filoviridae*)
- (-) ssRNA virus; genome cca 19k RNA basis
- GP exists in different forms,encoded by same RNA.
- EBOV RNA encodes only 7(8) proteins
- EBOV proteins are multi-functional in general. One protein could build different aggregates which has different function.
- ……

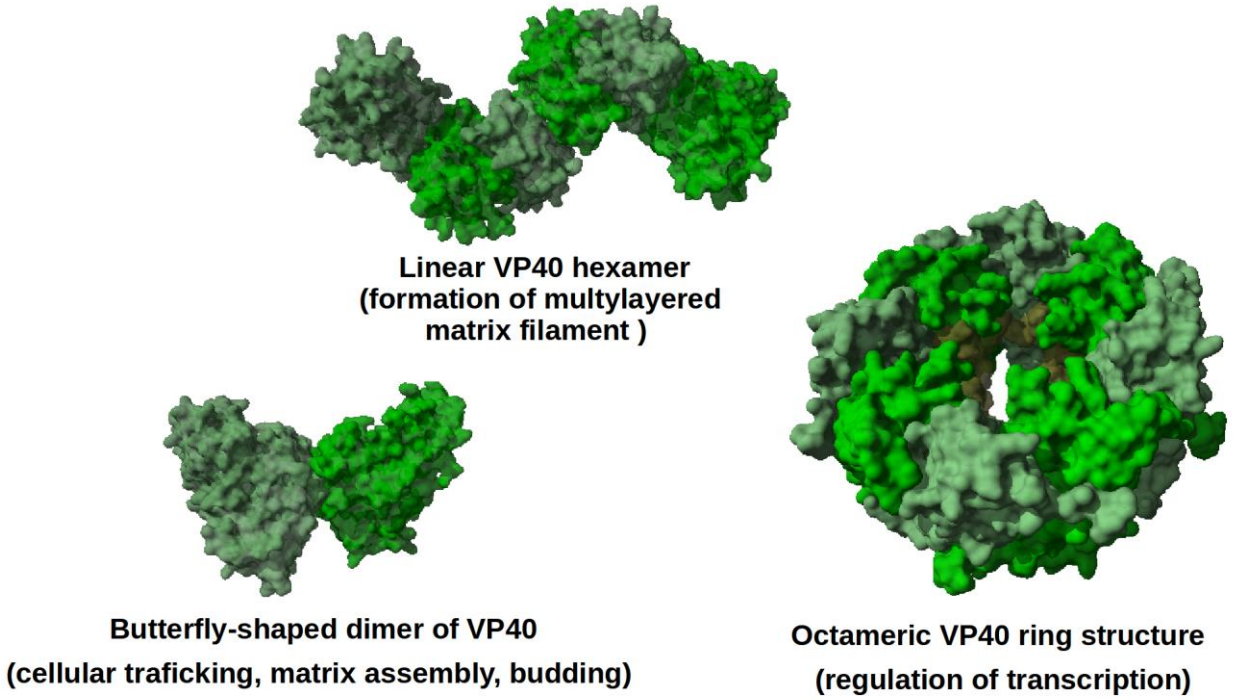
Structure of EBOV & its proteins



doi:10.2210/rcsb_pdb/mom_2014_10 by David Goodsell, Oct 2014

Multi-functionality of EBOV proteins

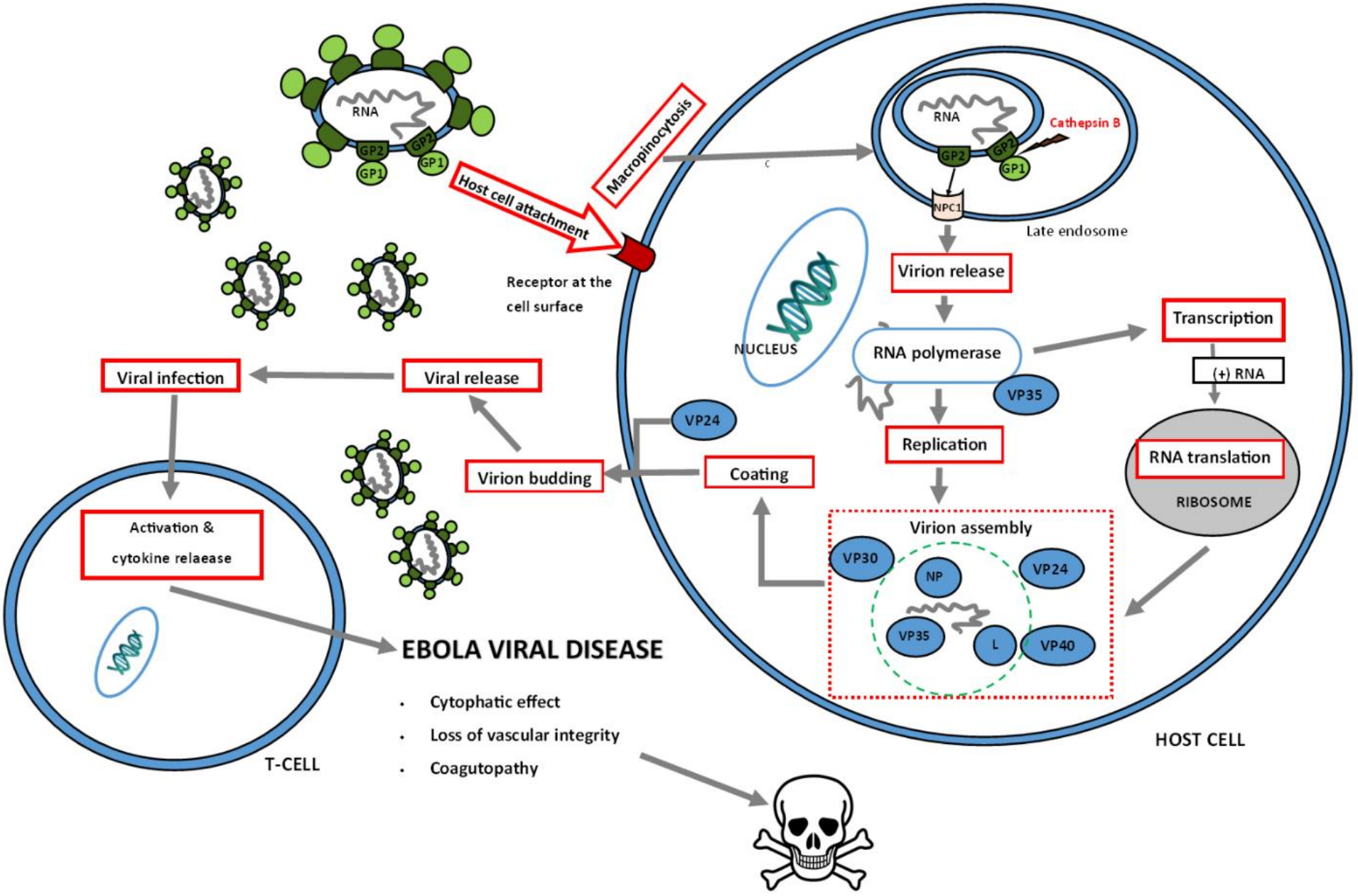
Only 7 proteins serve as building blocks and for “action” of EBOV. Ebola proteins aggregates to different superstructures which have different Functions in virus Life Cycle.



Typical example of multi-functionality of EBOV proteins is protein VP40, which serve as building block for viral assembly. VP40 as cyclo-octamer is involved in regulation of viral transcirption.

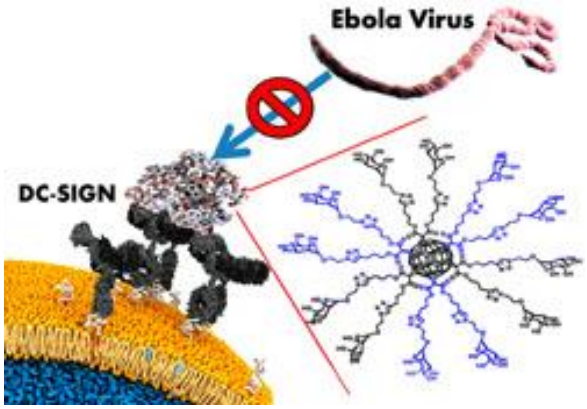
Bornholdt, Zachary A. *et al*. **Structural Rearrangement of Ebola Virus VP40 Begets Multiple Functions in the Virus Life Cycle,** Cell , Volume 154 , Issue 4 , 763 - 774

Live cycle of Ebola Virus

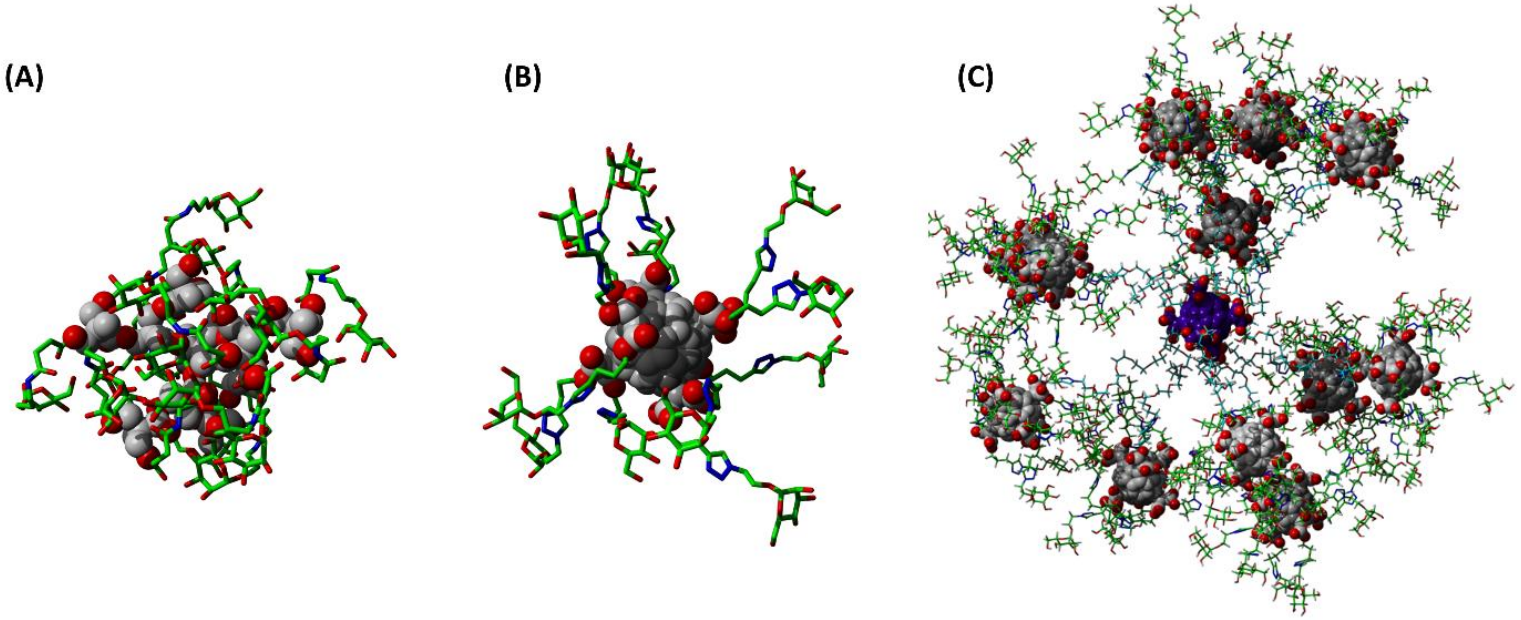


Live cycle of Ebola virus is quite complex. Possible targets and processes that can be used for drug design are in red.

Multivalency - Enhancing activity of EBOV inhibitors (Targeting host-cell receptors)



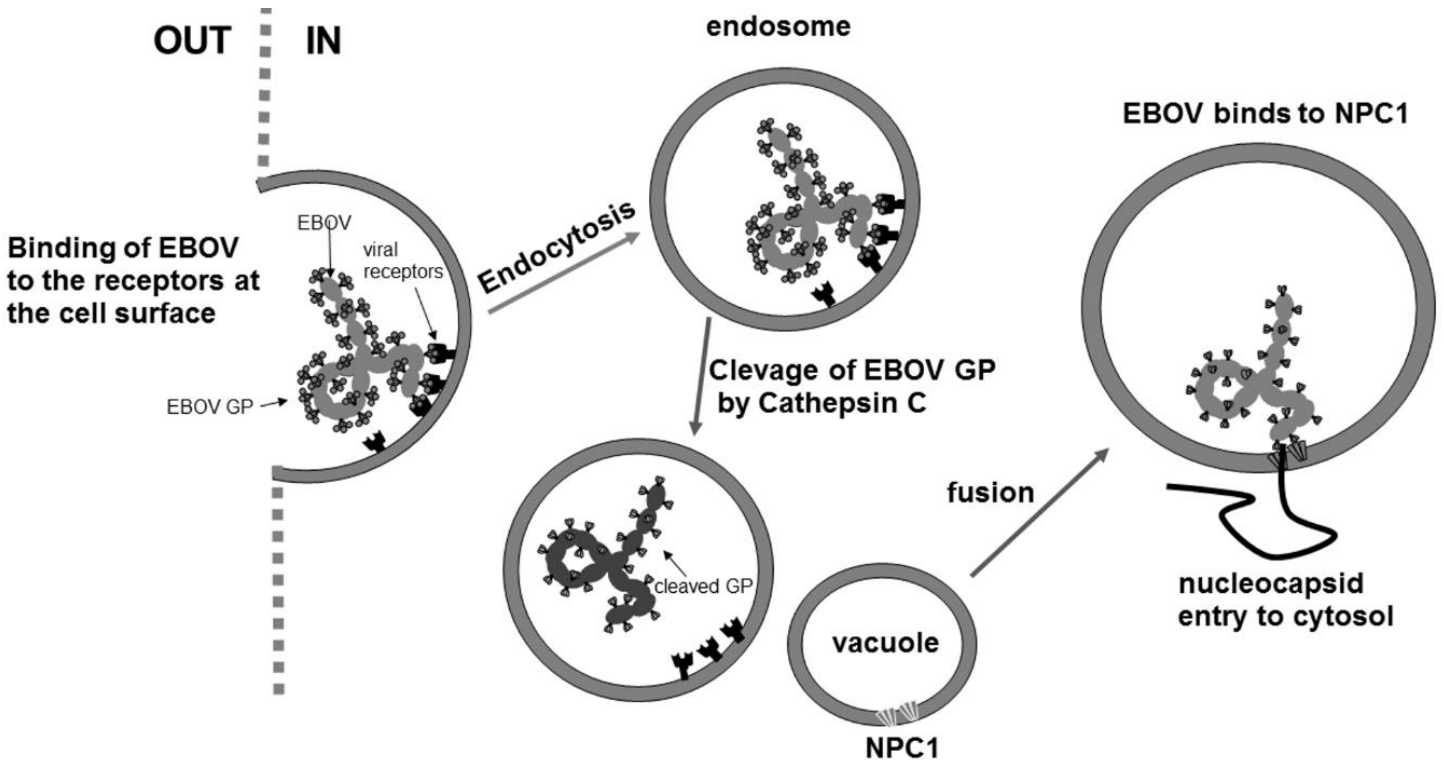
Attaching of the virus to host cell receptors (like DC-SIGN, TIM-1, etc) is first phase of EBOV “live cycle”. Ligand that binds to these human host receptors may prevent the virus to adhere at the surface of the cell.

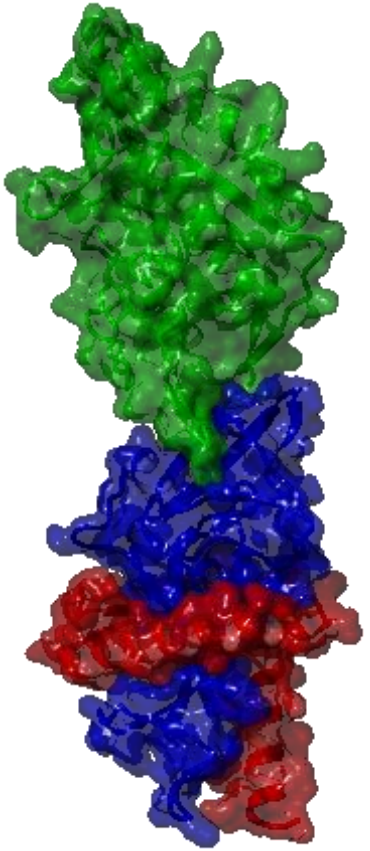


Dendrimeric glicoconjugates: (A) Boltorn-type (16 copies of mannose); (B) Glycodenro-fullerene (12 copies of mannose); (C) Tridecafullerene's superstructure decorated with 120 copies of mannose.

Munoz, *et al* **Synthesis of giant globular multivalent glycofullerenes as potent inhibitors in a model of Ebola virus infection** Nature Chemistry 8, 50–57 (2016), doi:10.1038/nchem.2387

Entry Event (Targeting NPC1 & Cathepsin C)





Ebola entry to host cell

Complex: NPC1:GPcl

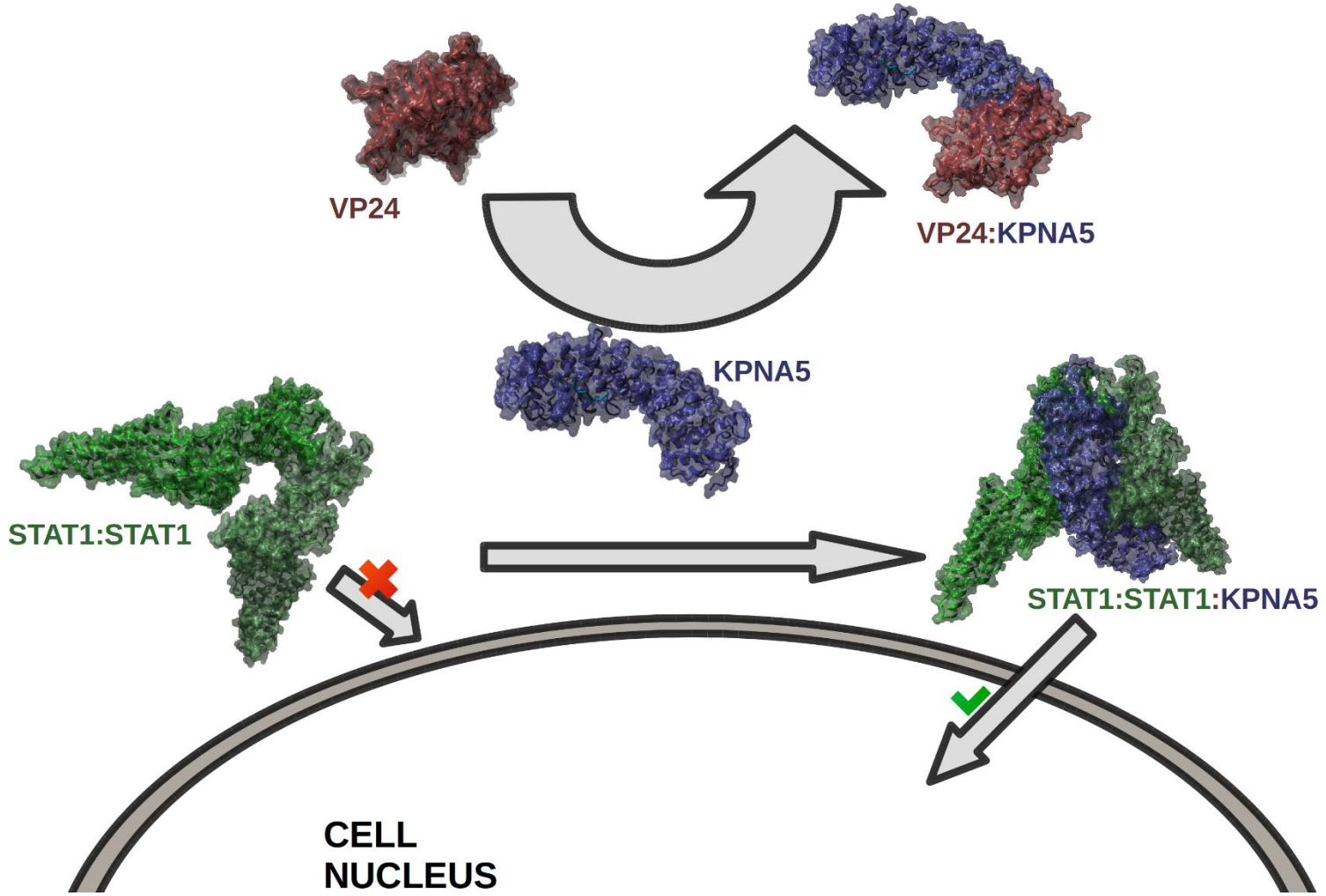
Two possible HOST targets in this process:

Cathepsin C -> this enzyme catalyze clevage of EBOV GP; if we block this enzyme EBOV can not escape from endosome.

NPC1 (Niemann Pick C1 receptor) -> NPC1 asists EBOV to escape to cytosol. Hidnering interaction between NPC1 and GPcl (cleaved GP) with „ligand“ may prevent accumulation of EBOV in the host cell.

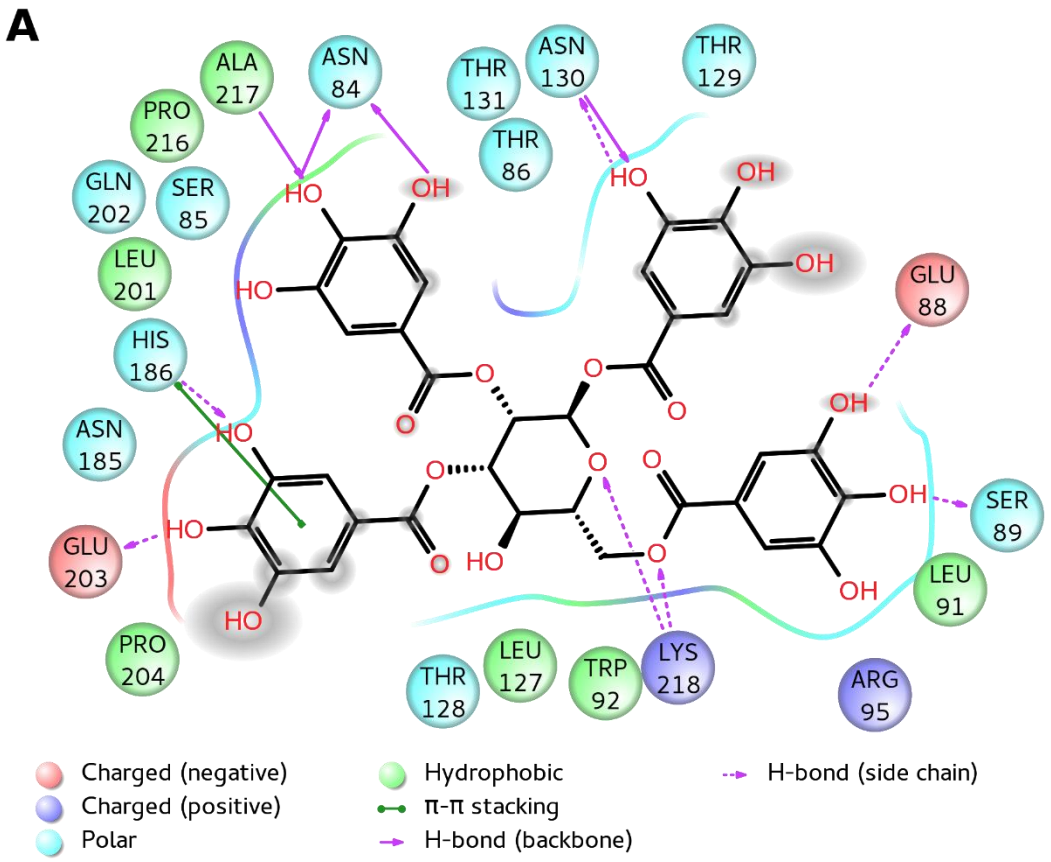
W.Han et. al Ebola Viral Glycoprotein Bound to Its Endosomal Receptor Niemann-Pick C1, Cell, 164 (2016) 258-268.

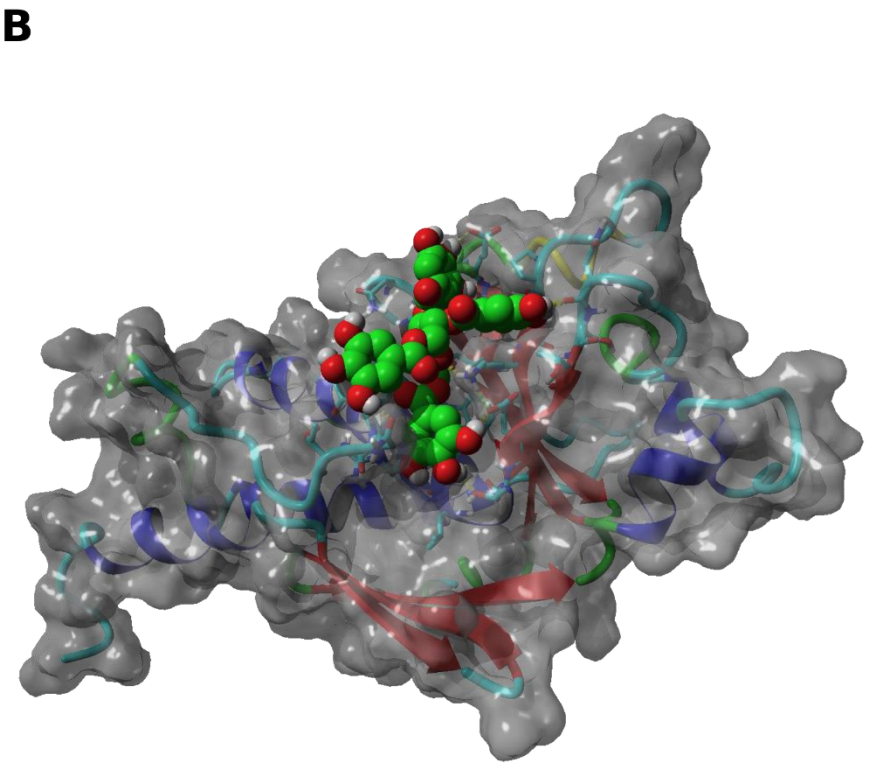
VP24 inhibits interferon induced cell response



VP24 binds to KPNA5 more than 100 fold tightly than STAT1:STAT1. STAT1:STAT1 can not enter to cell nucleus, where is acting as an activator of transcription. Host cell can not activate immune response via ….

Docking of 1,2,3,6-TGG to the active site of VP24



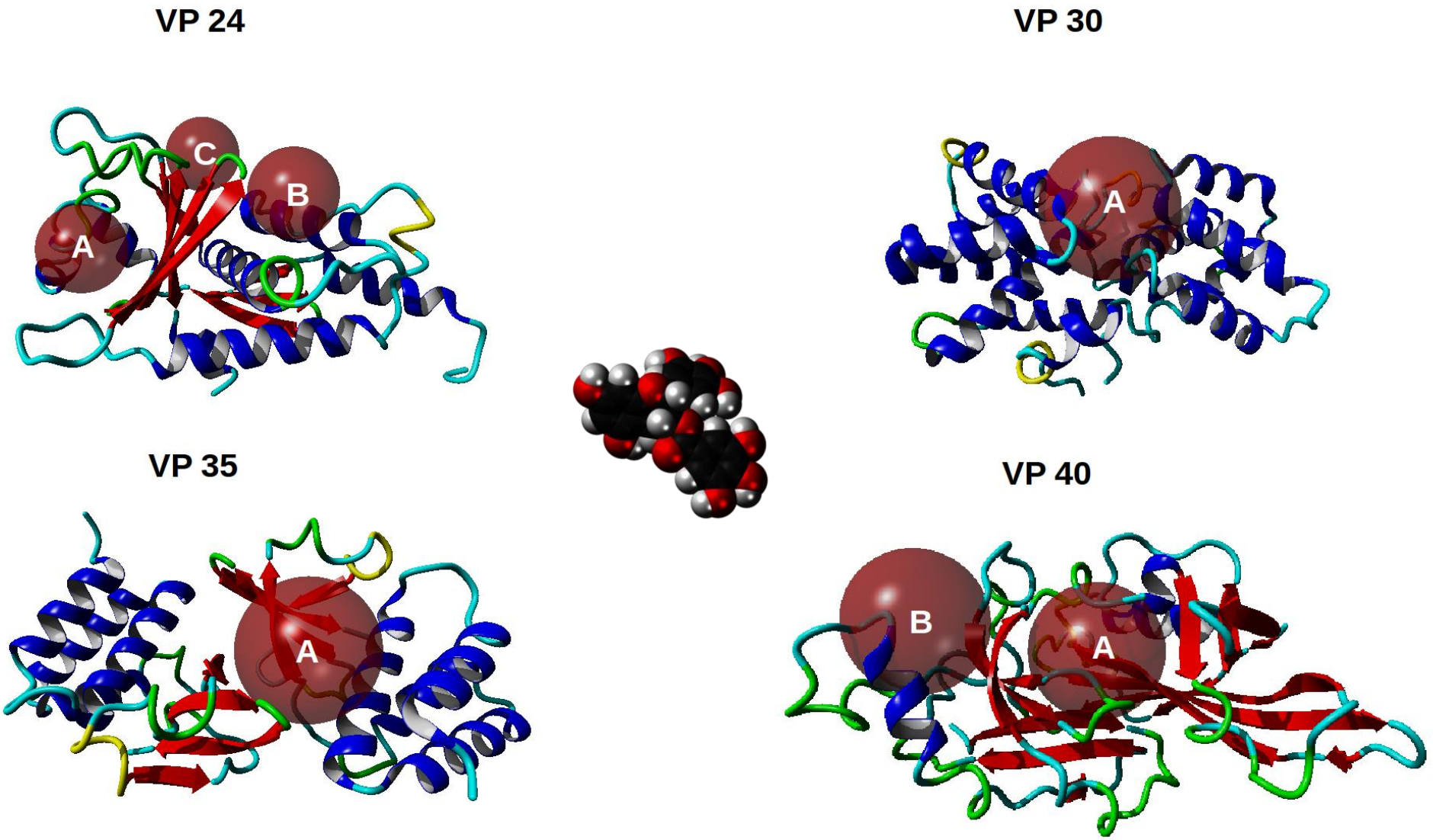


IFD GlideXP Score = -14,5 kcal/mol

TGG is compound could hinder interaction between karyopherin and VP24, and such as limit interferon inhibitory action of VP24.

Sebastian Pleško, Helena Volk, Miha Lukšič, Črtomir Podlipnik *In Silico Study of Plant Polyphenols' Interactions with VP24-Ebola Virus Membrane-associated Protein.* Acta Chim Slov 2015 ;62(3):555-64.

Hot-spots of various EBOV proteins obtained with Blind Docking



“Hot-spots” were identified by blind docking protocol using EGCG as a ligand. YASARA was used as an interface for running Autodock/Vina. The search was done by selecting coarse grid in extension 5 A from the protein. Binding sites were proposed by analysis of clusters after 100 independent runs of AD/Vina.

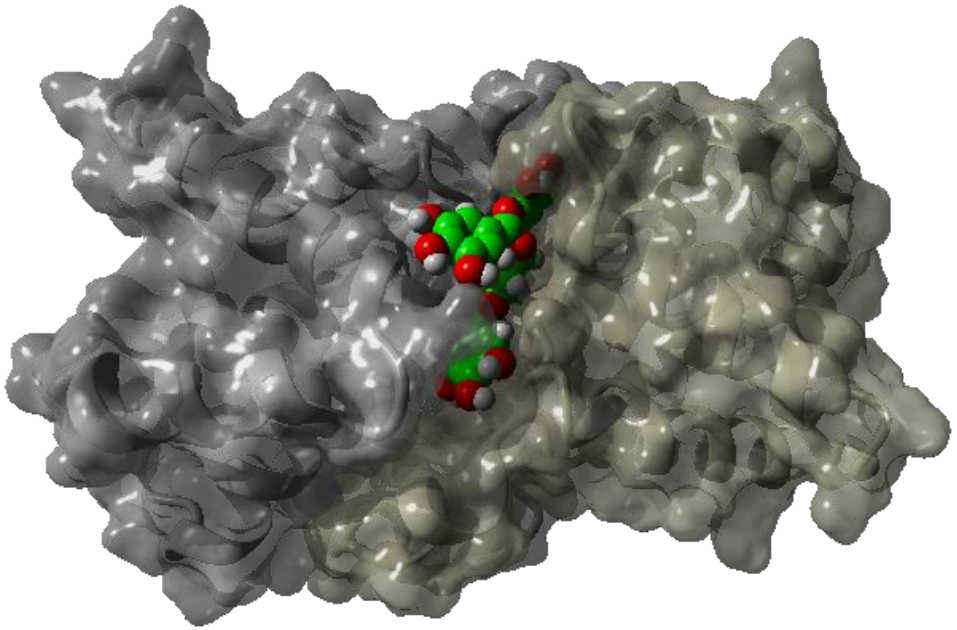
“Ensemble” docking experiment – 25 ligands x 7 models (YASARA/Vina)

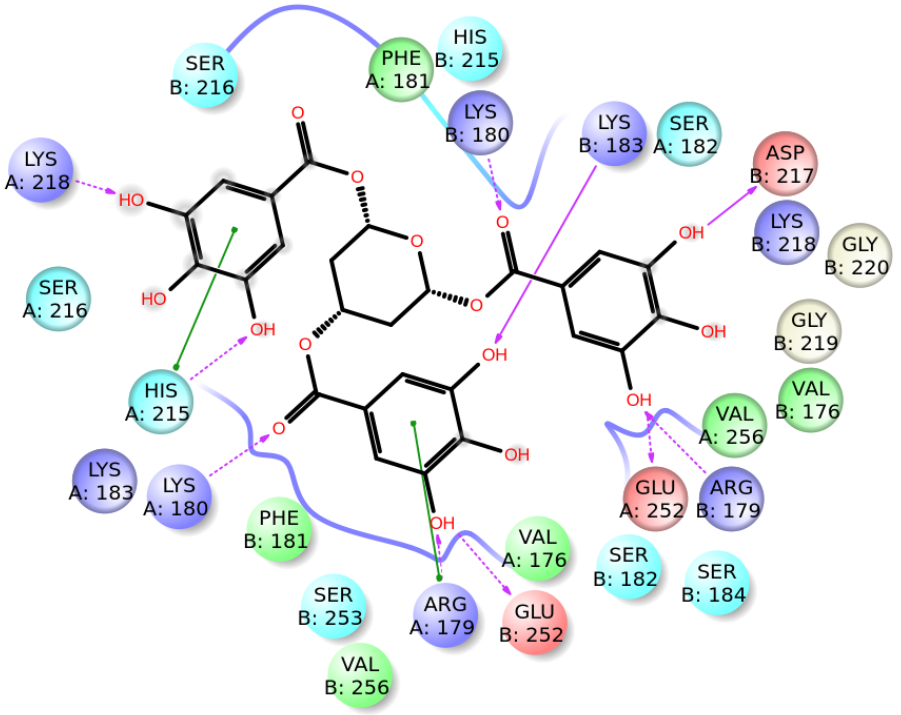
LIGAND	VP24_A	VP24_B	VP24_C	VP30	VP35	VP40_A	VP40_B
I01	-6.48	-6.74	-6.95	-9.04	-6.34	-8.74	-8.80
I02	-6.23	-6.45	-6.92	-8.79	-6.27	-8.52	-8.53
I03	-6.76	-7.01	**-7.53**	**-9.05**	**-8.30**	-9.75	-9.74
I04A	-6.73	-6.50	-6.93	-8.88	**-8.24**	-9.22	-9.25
...	...	...	...	...	...	...	...
L01	-6.76	-6.93	**-7.39**	-8.75	-7.14	-10.24	-10.27
L02	-6.21	-6.84	-6.52	-8.36	-7.17	-9.50	-9.60
L03	**-7.36**	**-7.04**	-7.05	**-9.65**	-7.83	**-10.95**	-10.03
...	...	...	...	...	...	...	...
L07	-6.80	-6.93	-7.39	-8.75	-7.15	-10.25	**-10.27**
L08	-6.04	-6.32	-6.71	-8.30	-6.46	-8.23	-8.23
L09	-6.92	**-7.15**	-6.82	-7.91	-7.99	**-10.79**	**-10.70**

Models of EBOV proteins (VP24, VP30, VP35 and VP40) have been used for “Ensemble” docking.

Results has been taken from student's work.

Docking small library to VP40 (best scored pose)





YASARA/AD-Vina Score =-10.95 kcal/mol

The best pose & score from “Ensemble docking” of small library of natural compounds.

Docking were prerformed with YASARA / AD-Vina

VSW of NuBBE Database to ensamble of targets based on EBOV proteins

NuBBE Database – virtual data base of compounds from Brasilian plants (currently cca 1700 compounds)

Settings of VSW protocol from Schrodinger:

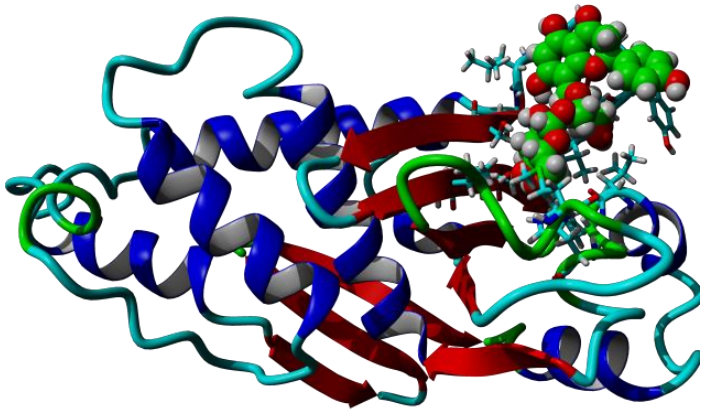
-> Ligand preparation with LigPrep

-> For all compounds Glide HTVS score were obtained

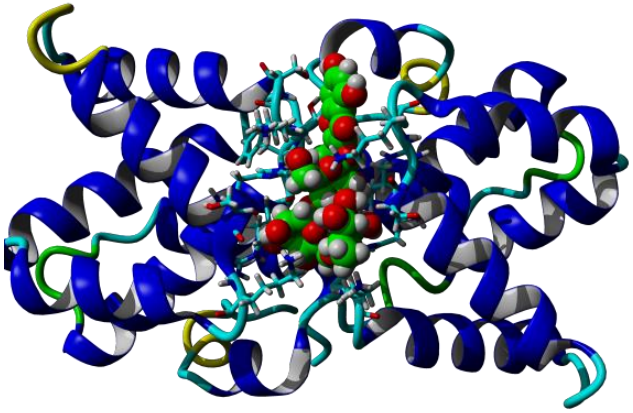
-> 20 % best Glide HTVS scored compounds went to next „Glide SP“ phase

-> Top 25 „Glide SP“ scored compounds were rescored using Glide XP

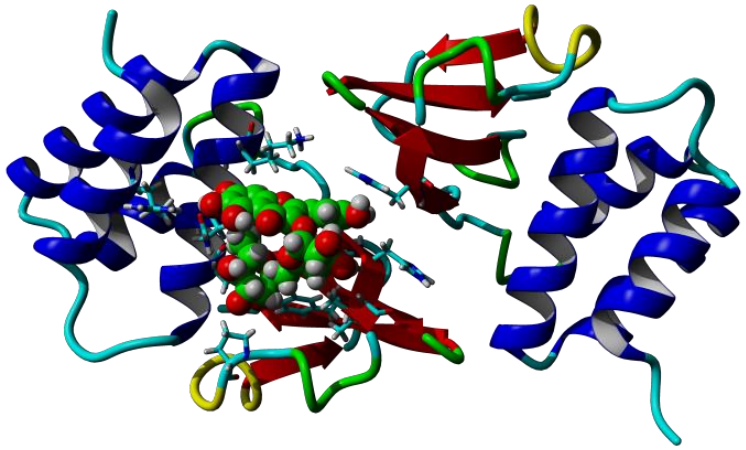
Best poses after VSW of NuBBE database



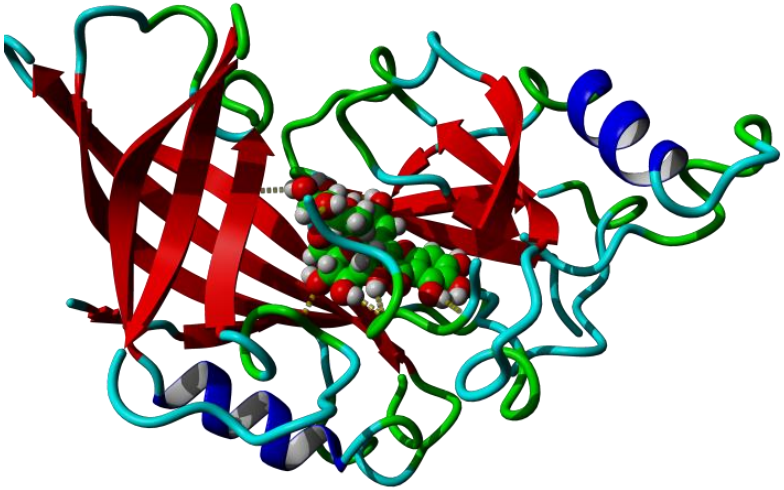
VP24_NuBBE.63 (GlideXP = -13,3 kcal/mol)



VP30_NuBBE.286 (GlideXP = -11,7 kcal/mol)

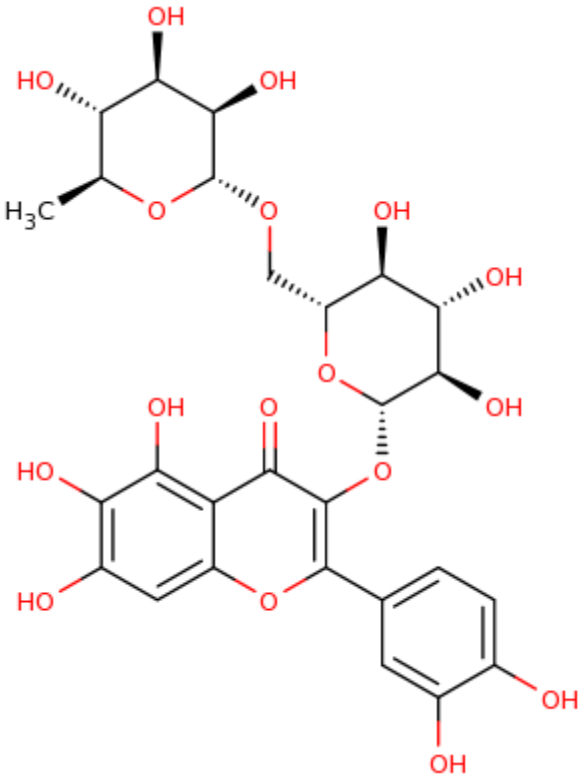


VP35_NuBBE.283 (GlideXP = -11,0 kcal/mol)



VP40_NuBBE.283 (GlideXP = -17,1 kcal/mol)

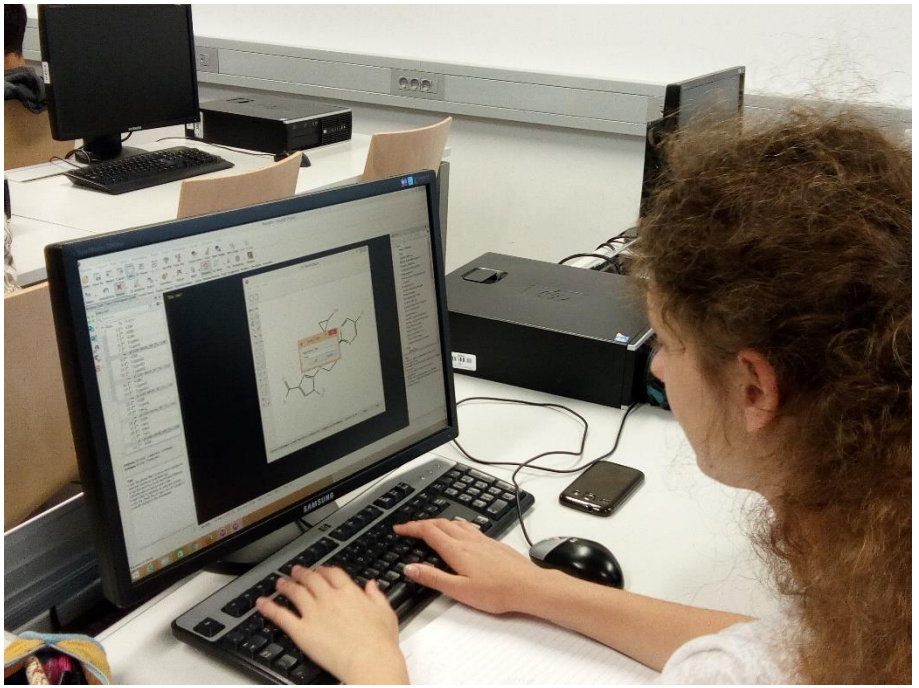
NuBBE.283 (6-hydroxy-rutin)



Isolated from plant Rubiaceae Chimarrhis turbinata

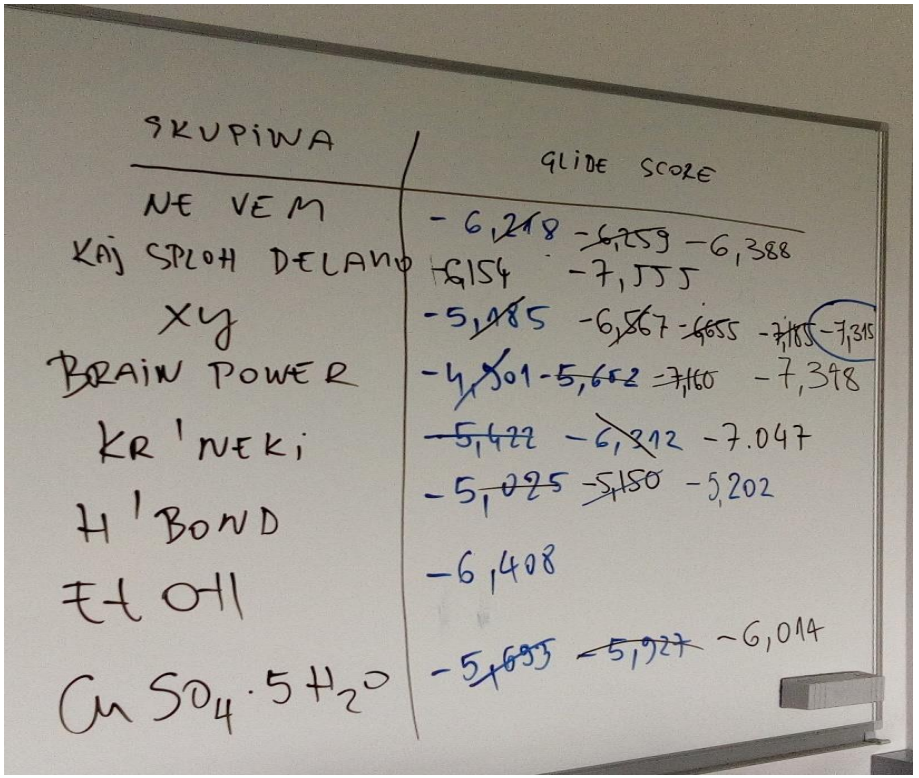
The best glide score for targets VP35 & VP40

Pedagogical Experiment
United High School Students Against EBOLA



Inspired by idea of FoldIt (http://www.fold.it) we organized a Summer School workshop where High School Students (without any extra knowledge about molecular modeling) compete to find the best „scored“ ligand for target EBOV VP35. They have used Schrodinger Suite 2016 (Thnx for additional lic.) for performing all modelling tasks.

Pedagogical Experiment
United High School Students Against EBOLA



After two hours of „Fight“ we got a winner of the competition with winning molecule.

•The future idea is to organize similar competition „Online“ with automatical collecting results (Web Server Application ?)

Thank you !