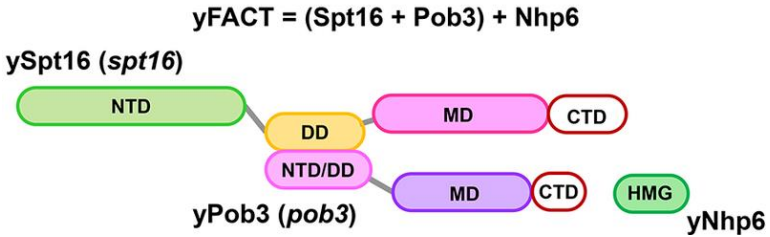
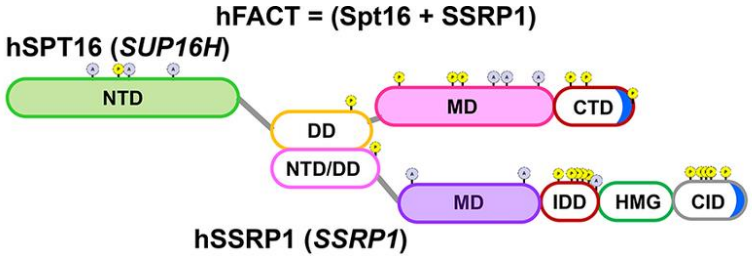
Comparison of nucleosome unfolding by yeast and human FACT: electron microscopy analysis

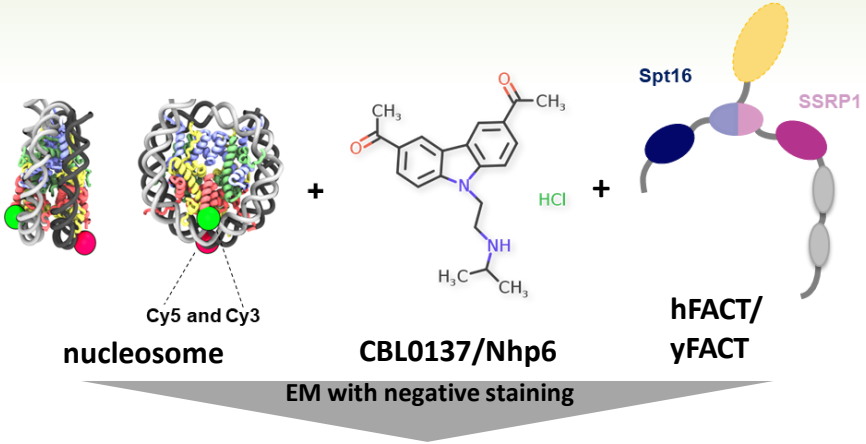
Sivkina A. [1#*], Volokh O.[1#], Karlova M. [1#], Sokolova O. [1,2], Studitsky V. [1,3]
1. Biology Faculty, Lomonosov Moscow State University, 119992 Moscow, Russia
2. Department of Biology, Shenzhen MSU-BIT University, Shenzhen, China Guangdong Province 518172, China
3. Fox Chase Cancer Center, Philadelphia, PA 19111, USA
- Equal authorship
* anastasiia.sivkina@gmail.com





Yeast FACT (yFACT) **VS** **Human FACT (hFACT)**

Motivation and Aim: FACT (facilitates chromatin transcription) is a histone chaperone that participates in nucleosome removal and reassembly during transcription and replication. Previously we have shown that yeast FACT dramatically alters the nucleosome structure without ATP hydrolysis, but the extent of these alterations depends on the presence of HMGB domain-containing protein Nhp6.
hFACT requires DNA-intercalators curaxins for nucleosome unfolding; presumably curaxins destabilizes nucleosomes and thus facilitates FACT-nucleosome interaction. Nevertheless, the detailed mechanism of this process is still unclear.



Methods and Algorithms: We used mononucleosomes assembled on the 603 Widom positioning sequence. Complexes of FACT with the nucleosome were formed in the presence of 0.1 μM FACT,0.1 μM core chicken nucleosomes, 0.5 nM fluorescently labeled core nucleosomes N35/112 and 5 μM CBL0137 (for human FACT) or 10 μM Nhp6 protein (for yeast FACT). For transmission electron microscopy (TEM) analysis samples were applied to the carbon-coated glow-discharged copper grid (Ted Pella, USA) immediately after preparation, and stained for 30 sec with 1% uranyl acetate. Grids were studied in JEOL 2100 TEM (JEOL) microscope operated at 200 kV at low-dose conditions. Single particles coordinates collected by the neural network were imported in RELION2.1 software; all further 2D-processing, analysis and CTF-correction were performed using RELION2.1 software.

yFACT+Nhp6+nucleosome

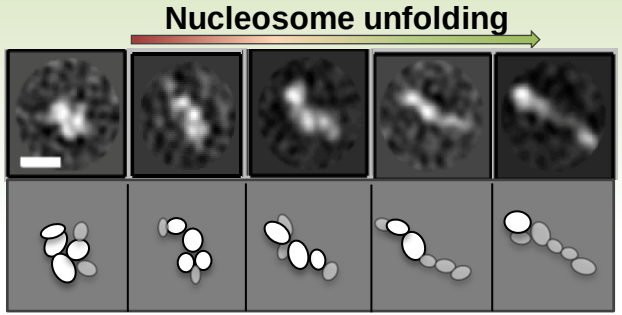


Fig.1. Representative 2D class averages of FACT:Nhp6:nucleosome complexes with different distances between edges of the complex are arranged to show the proposed sequence of events during nucleosome unfolding by FACT:Nhp6. Scale bar: 10 nm.

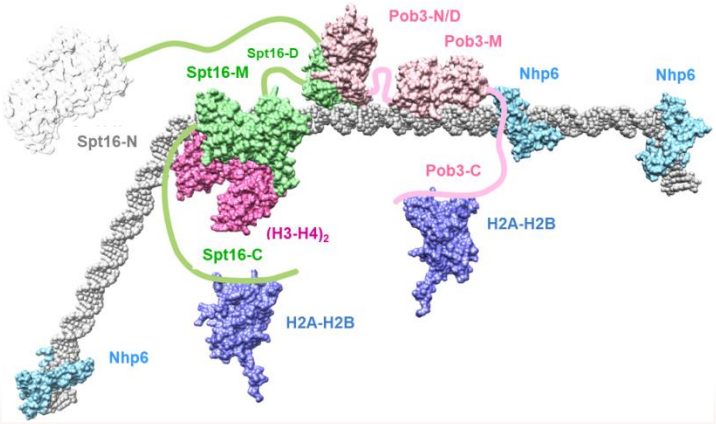


Fig.2. The proposed structure of the unfolded FACT:Nhp6:nucleosome complex.

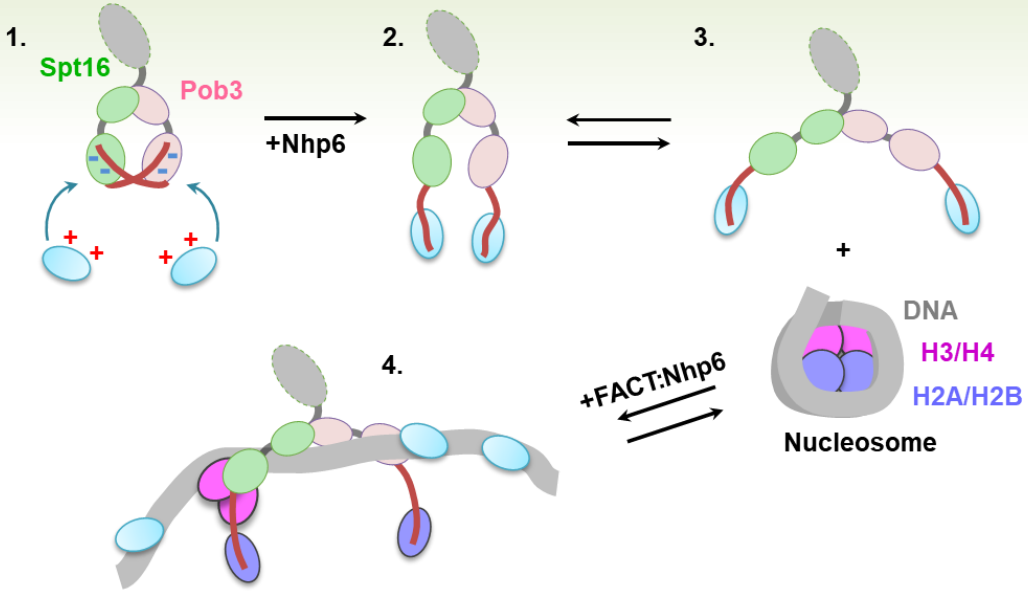


Fig. 3. Model of nucleosome unfolding by yeast FACT.

hFACT+CLB0137+nucleosome

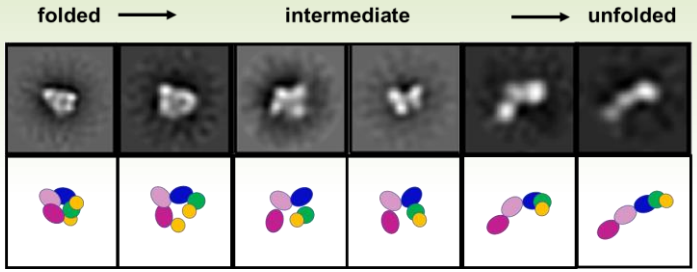


Fig. 4. Nucleosome unwrapping by FACT in the presence of CBL0137. Characteristic 2D class averages of FACT-nucleosome complexes in the presence of CBL0137. The complexes are arranged to show the proposed sequence of events during nucleosome unfolding by FACT

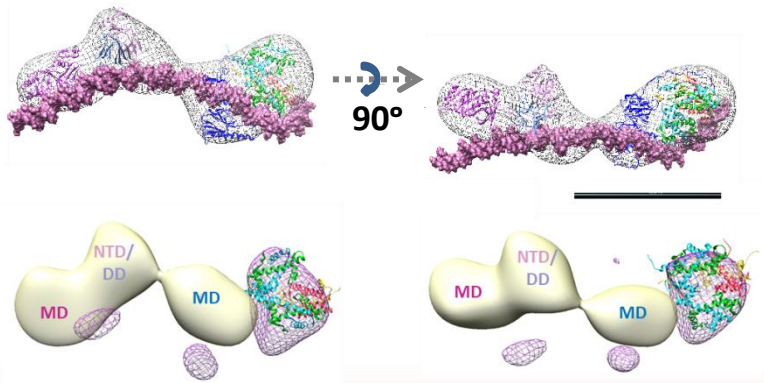


Fig. 5. Structures of folded and unfolded FACT-nucleosome complexes formed in the presence of curaxin CBL0137.

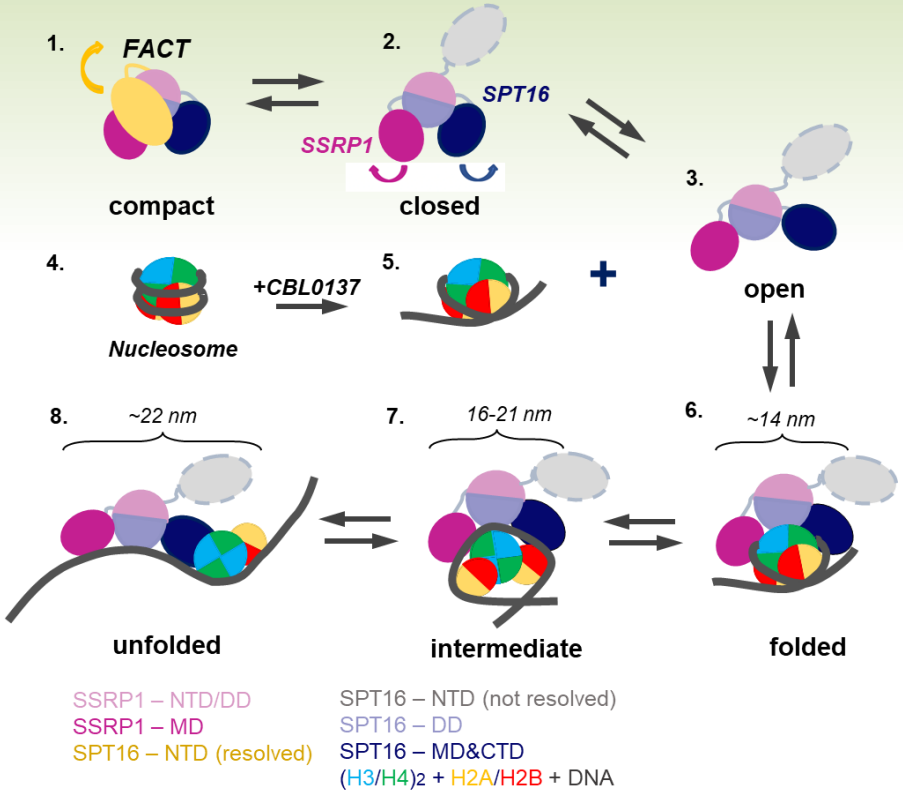


Fig. 6. Model of nucleosome unfolding by hFACT in the presence of curaxin CBL0137.

Results:

Here using TEM we studied human FACT (hFACT) and yeast FACT (yFACT) flexibility alone and in complexes with nucleosomes. All studied complexes are highly flexible and adopt broad ranges of configurations. DNA-binding protein Nhp6 binds to the C-terminal tails of both yFACT subunits and induces formation of more open FACT complexes, thus altering the structures of FACT and the nucleosomes and facilitating nucleosome unfolding.

Multiple closed and open conformations were also demonstrated for nucleosome-free hFACT. The open conformations of FACT become predominant during curaxin-induced nucleosome unfolding involving multiple intermediates. We demonstrated that both yFACT and hFACT flexibility facilitates FACT-dependent nucleosome unfolding that occurs similarly for yFACT and hFACT, resulting in formation of nearly linear, extensively unfolded structure.

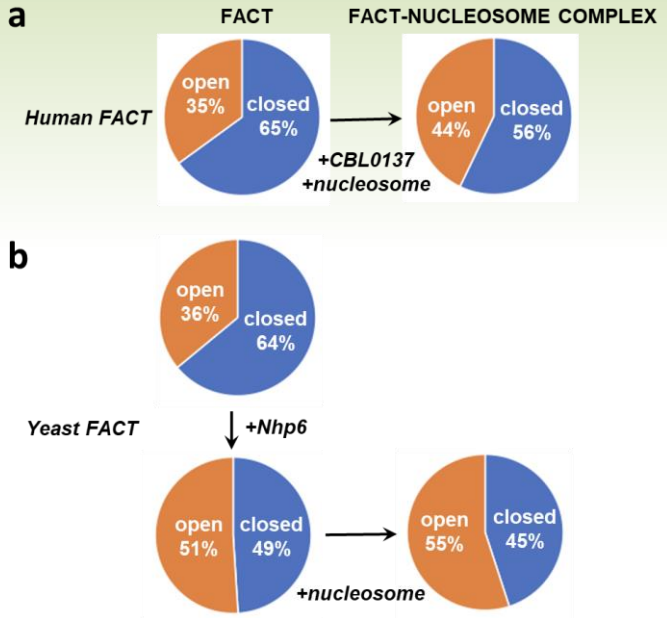


Fig.7. Conformations of human (a) and yeast (b) FACT in solution and in FACT-nucleosome complexes.
To allow easier comparison between different samples, all complexes containing compact and open conformations of FACT were counted as closed and open complexes, respectively.

Conclusion: The data suggest that the process proceeds through a series of energetically similar intermediate structures, ultimately leading to an extensively unfolded form. We proposed FACT-dependent nucleosome unfolding pathway based on a large number of potential intermediates revealed by electron microscopy.

Acknowledgements:
This work was supported by the Russian Science Foundation (#19-74-30003). Electron microscopy was performed on the Unique equipment setup “3D-EMC” of Moscow State University, Department of Biology.

Explore more about FACT:

1. Valieva, M.E., et al., *Large-scale ATP-independent nucleosome unfolding by a histone chaperone.* Nat Struct Mol Biol, 2016. **23**(12): p. 1111-1116.
2. Chang, H.W., et al., *Histone Chaperone FACT and Curaxins: Effects on Genome Structure and Function.* J Cancer Metastasis Treat, 2019. **5**.
3. Sivkina, A.L., et al., *Electron microscopy analysis of ATP-independent nucleosome unfolding by FACT.* Commun Biol, 2022. **5**(1): p. 2.
4. Volokh O.I., Sivkina A.L., et al. *Mechanism of Curaxin-dependent Nucleosome Unfolding by FACT.* Bioarxiv preprint, 2022. **doi:** 10.1101/2022.05.10.491363

Feel free to contact me: ***anastasiia.sivkina@gmail.com*** ☺