Poster

Nanoscreening of Topoisomerase Inhibition by Lamellarin D

Daniel Pla,[a, b, c] *Andy Sischka*,[c] *Xavier Fernández-Busquets*,[d] *Fernando Albericio*,[a, b] *Mercedes Álvarez*,[a, b] *Dario Anselmetti*,[c,*]

a: Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain; b: CIBER-BBN Networking Centre on Bioengineering, Biomaterials and Nanomedicine; c: Experimental Biophysics and Applied Nanosciences, Bielefeld University, D-33615 Bielefeld, Germany; d: Biomolecular Interactions Team (BIT), Nanobioengineering Group, Institute for Bioengineering of Catalonia, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona.
daniel.pla@irbbarcelona.org, *Dario.Anselmetti@physik.uni-bielefeld.de*

From the active anti-cancer chemotherapy research lines, topoisomerases [1] stand in a high relevant topic of interest due to the specificity properties of the Topo-DNA complex [1, 2]. DNA handling enzymes such as topoisomerases represent a promising and direct effector against tumour proliferation, and a selective weapon against uncontrolled cellular growth. Topoisomerases, as several biologically important macromolecules, undergo mechanical motions that are essential to their function. Therefore, robust, powerfull and highly sensitive biophysical applications [3-5] are needed for micromanipulation techniques with high resolution at the single-molecule level. Understanding the mechanism of action of the enzyme in the presence of its inhibitors is a major requirement for future clinical development of important therapeutic agents. We applied atomic force

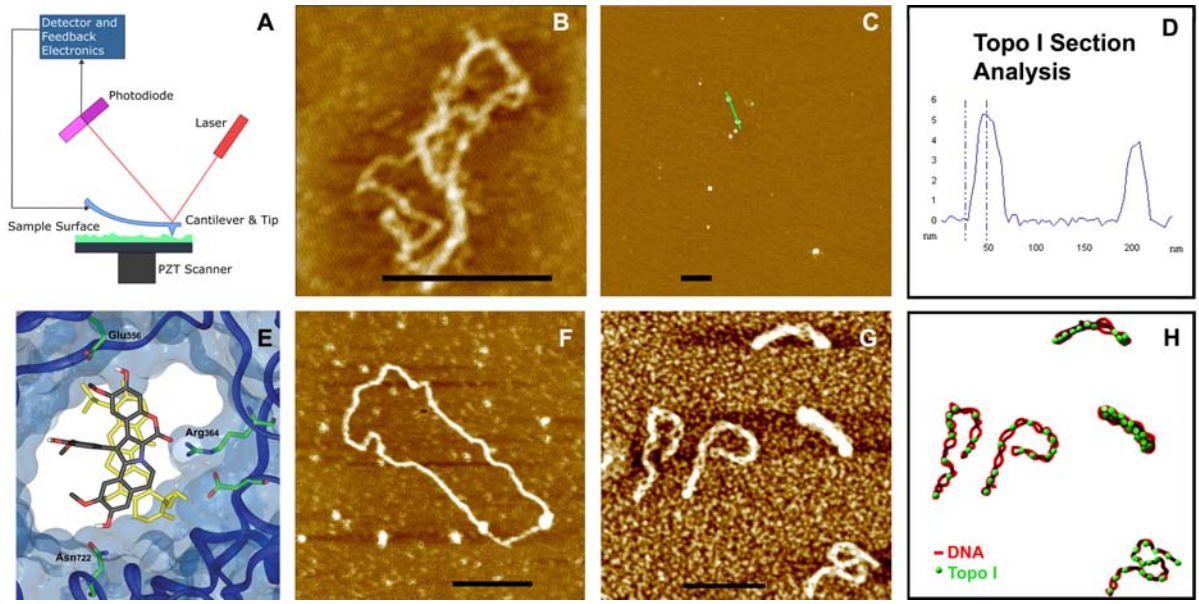

Fig. (1) **(A)** Schematic AFM nanoscope. **(B)** AFM image of supercoiled DNA. **(C)** AFM image of Topo I. **(D)** Topo I section analysis. **(E)** Crystallographic model of topotecan–DNA–topoisomerase (PDB 1k4t) featuring LAMD superimposed in the active site. **(F)** AFM image of relaxed DNA after reaction with Topo I. **(G)** AFM image of LAMD-Topo I- DNA ternary complexes. **(H)** Diagrammatic representation of LAMD-Topo I- DNA ternary complexes obtained by AFM. bars represent 200 nm

microscopy (AFM), a useful single molecule technique in order to identify and investigate the biophysical mechanisms that might help to develop a topoisomerase inhibition nanoscreening.

The widely known marine alkaloid Lamellarin D (LAMD) [6-9], belonging to a group of anticancer agents with the singular mechanism of poisoning DNA-Topo I phosphotyrosyl complexes [8, 10], has been used for the inhibition of Topo I. Interaction of the drug with a DNA-Topo I can produce a stable, cytotoxic complex [11] *(fig. 1E)* that inhibits post-cleavage DNA religation processes [2]. *Fig. 1B* demonstrates an AFM (*fig. 1A)* image of a supercoiled DNA-plasmid showing a plectonemic structure. Characterization of Topo I *(fig. 1C-D)* was carried out where an average size of 5 nm height and 19 nm width (FWHW) could be determined. Following 30 min of topo I activity, the AFM images showed that all of the initially supercoiled 3400 bp DNA molecules were converted to the fully or partially relaxed forms *(fig. 1F)*. The LAMD inhibition of the topoisomerase activity could be readily monitored by imaging of plectonemic DNA structures conjugated with enzyme attachements in the presence of LAMD *(fig. 1G-H)*. These data are in full accordance with previously reported biological [2] and computational [8, 10] studies regarding its mode of action.

These interesting insights provided by single molecule applications and biophysical nanoscreening methodologies will certainly be decisive to the better knowledge of the mechanism of action how potential topoisomerase inhibitors like LAMD can be investigated and screened at the nanometer scale and pN level for pharmacological applications.

References:

[1] Cortés, F.; Pastor, N.; Mateos, S.; Domínguez, I., Expert Opin. Ther. Patents **17**, (2007) 1.
[2] Dias, N.; Vezin, H.; Lansiaux, A.; Bailly, C., Top. Curr. Chem. **253** (2005) 89.
[3] Neuman, K. C.; Lionnet, T.; Allemand, J.-F., Annu. Rev. Mater. Res. **37** (2007) 33.
[4] Greenleaf, W. J.; Woodside, M. T.; Block, S. M., Annu. Rev. Biophys. Biomol. Struct. **36** (2007) 171.
[5] McCauley, M. J.; Williams, M. C., Biopolymers **85** (2006) 154.
[6] Vanhuyse, M.; Kluza, J.; Tardy, C.; Otero, G.; Cuevas, C.; Bailly, C.; Lansiaux, A., Cancer Lett. **221** (2005) 165.
[7] Bailly, C., Curr. Med. Chem.: Anti-Cancer Agents **4** (2004) 363.
[8] Facompré, M.; Tardy, C.; Bal-Mayeu, C.; Colson, P.; Pérez, C.; Manzanares, I.; Cuevas, C.; Bailly, C., Cancer Res. **63** (2003) 7392.
[9] Pla, D.; Marchal, A.; Olsen, C. A.; Francesch, A.; Cuevas, C.; Albericio, F.; Alvarez, M., J. Med. Chem. **49** (2006) 3257.
[10] Marco, E.; Laine, W.; Tardy, C.; Lansiaux, A.; Iwao, M.; Ishibashi, F.; Bailly, C.; Gago, F., J. Med. Chem. **48** (2005) 3796.