Single-molecule force spectroscopy on a G protein-coupled receptor

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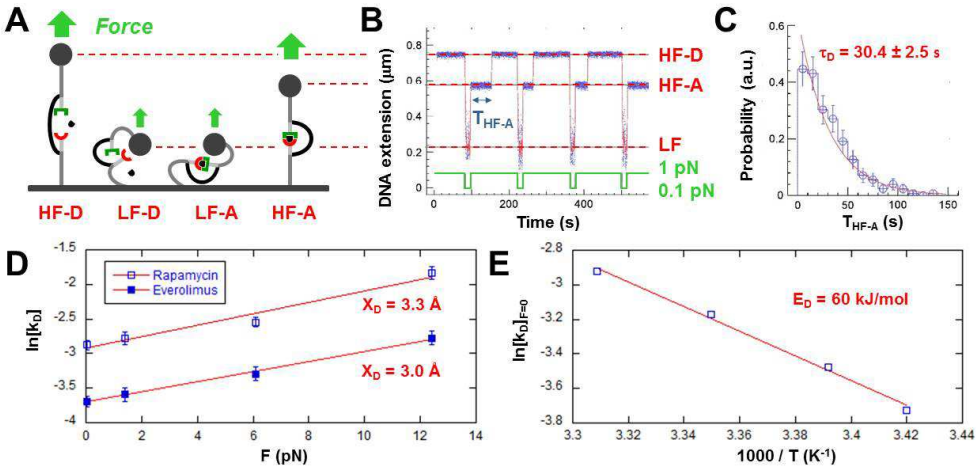
Our team has devised a modular scaffold made of DNA and dedicated to single-molecule studies. It allows one to easily manipulate interacting biomolecules that have been engrafted at its tips, as with forceps.[1-3] Using this construct, called jDNA, we can pull on the formed complex with magnetic tweezers and follow in real-time the disruption of the interaction (see Figure). Then, from the force and temperature dependence of the dissociation rate constant one can reconstruct the energy landscape towards dissociation, especially if different pulling directions are studied.[2] Such strategy as already been applied with success to the interaction between the FKBP12 and FRB proteins, which is mediated by rapamycin, a drug used to prevent transplant rejection and to fight against cancer.[2] Investigations on systems involved in synapse plasticity, immunotherapy, and viral infection are underway.

G protein-coupled receptors (GPCR) are important in pharmacology since they represent 34 % of the total number of targets. However, because they are membrane proteins they have been poorly studied by single-molecule force spectroscopy (SMFS). It is indeed difficult to produce them in a solubilized form and, even if one succeeds, the obtain objects are quite fragile. On the other hand, jDNA place the investigated biomolecules far from any surface and enable one to easily discriminate between specific and non-specific interactions. Therefore, we here propose to realize the first demonstration of SMFS on purified GPCR, the ultimate goal being to reconstruct the energy landscape towards dissociation in presence of the various drugs that modulate the activity of these proteins in vivo. This information in hand we hope to be able to optimize the kinetic properties of the considered ligands and lower their side effects.

A first part of the internship will be dedicated to the expression and purification of the GPCR imbedded in a nanodisc. It will be achieved in collaboration with a team of specialized biochemists at IBPC.[4-7] Then, the obtained objects will be engrafted on jDNA, in front of some of their ligands, and SMFS experiments will be carried out at IBENS.



Use of jDNA forceps to study the dissociation of the ternary FRB • rapamycin • FKBP12 complex. (A) *The two partner proteins (red and green) are assembled onto the DNA scaffold made of two shanks (dark grey), two tips (light grey), and a leash (black); the rapamycin drug is in solution. When a high force, HF, is applied to the magnetic bead the associated A state and the dissociated D state exhibit different extensions. When a low force, LF, is applied the associated and dissociated states can hardly be distinguished.* ***(B)*** *Time trace of the magnetic bead position (blue line) upon force-cycling (green line). For each force-cycle the dwell-time before rupture, T_{HF-A}, is measured (if above the 0.1 s detection threshold).* ***(C)*** *Extraction of τ_D by mono-exponential fitting.* ***(D)*** *Determination of X_D and $\ln[k_D]_{F=0}$ by linear fitting of $\ln[k_D](F)$ at 25 °C. Additional data for the everolimus drug are displayed for comparison.* ***(E)*** *Determination of E_D by linear fitting of $\ln[k_D]_{F=0}(1/T)$.*

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