DEVELOPMENT OF SINGLE MOLECULE NANOBIOSENSORS FOR THE SEARCH OF ANTIBIOTIC AND ANTIMALARIAL COMPOUNDS

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The discovery of new bioactive compounds to treat microbial infections is an ever increasing need as a result of the constant evolution of resistance by the infectious agents, notably malaria and pathogenic bacteria. By using a nanotechnology approach based on Chemical Force Microscopy, a derivation of Atomic Force Microscopy that allows the measurement of interaction forces between single molecules, we are searching for enzyme inhibitors that are good candidates for the development of new antimalarials and antibiotics. The main advantage of this high-throughput screening approach is that by monitoring single molecule interactions novel inhibitory molecules can be detected that otherwise escape identification by current methods requiring high concentrations of the sought-after compound.

Our general strategy consists on the search for inhibitors of molecular interactions that are essential for the survival of the pathogenic agents. In this work, we have selected as metabolic target a biosynthetic route that is present in the malaria parasite and in most human pathogenic bacteria, but not in vertebrates, thus representing a potential source of highly specific antimalarials and antibiotics. The chosen enzyme/substrate pairs are immobilized on the atomic force microscope (AFM) tip and surface following chemical methods.

The substrate, a three-carbon compound, is immobilized by one of two alternative methods: an unspecific adsorption onto gold (overnight immersion of gold surfaces in aqueous solution of the substrate), or a specific linking of a thiol derivate of the substrate onto gold. Organic synthesis has been used to modify the substrate with a thiol linker. The resulting compound is expected to diminish steric hindrances that can hamper the access of the substrate to the active center of the enzyme, thus favoring an improvement of the interaction force to be detected. The enzymes are immobilized onto a carboxyl-terminated self-assembled monolayer formed on the gold-coated AFM tip using N-hydroxysuccinimide and N-ethyl-N'-(3-diethylaminopropyl) carbodiimide coupling of lysine side chains on the protein to the surface carboxyl groups [1]. Enzymes immobilized with this method usually maintain catalytic activity [2,3].

Force spectroscopy experiments are then performed in liquid medium, yielding force curves that indicate the existence of enzyme-substrate interactions (Figure 1). Different concentrations of buffers and cofactors will be tested to provide physiological conditions for an optimal interaction. Once the specificity of the interaction is established with the corresponding controls in the presence of soluble substrate and in the absence of one of the binding partners, natural product extracts and chemical libraries will be screened for their inhibitory activity. We will give preference to extracts from marine sponges, considering their metabolic diversity and their high content in cytotoxic compounds.

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References:

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Figures:

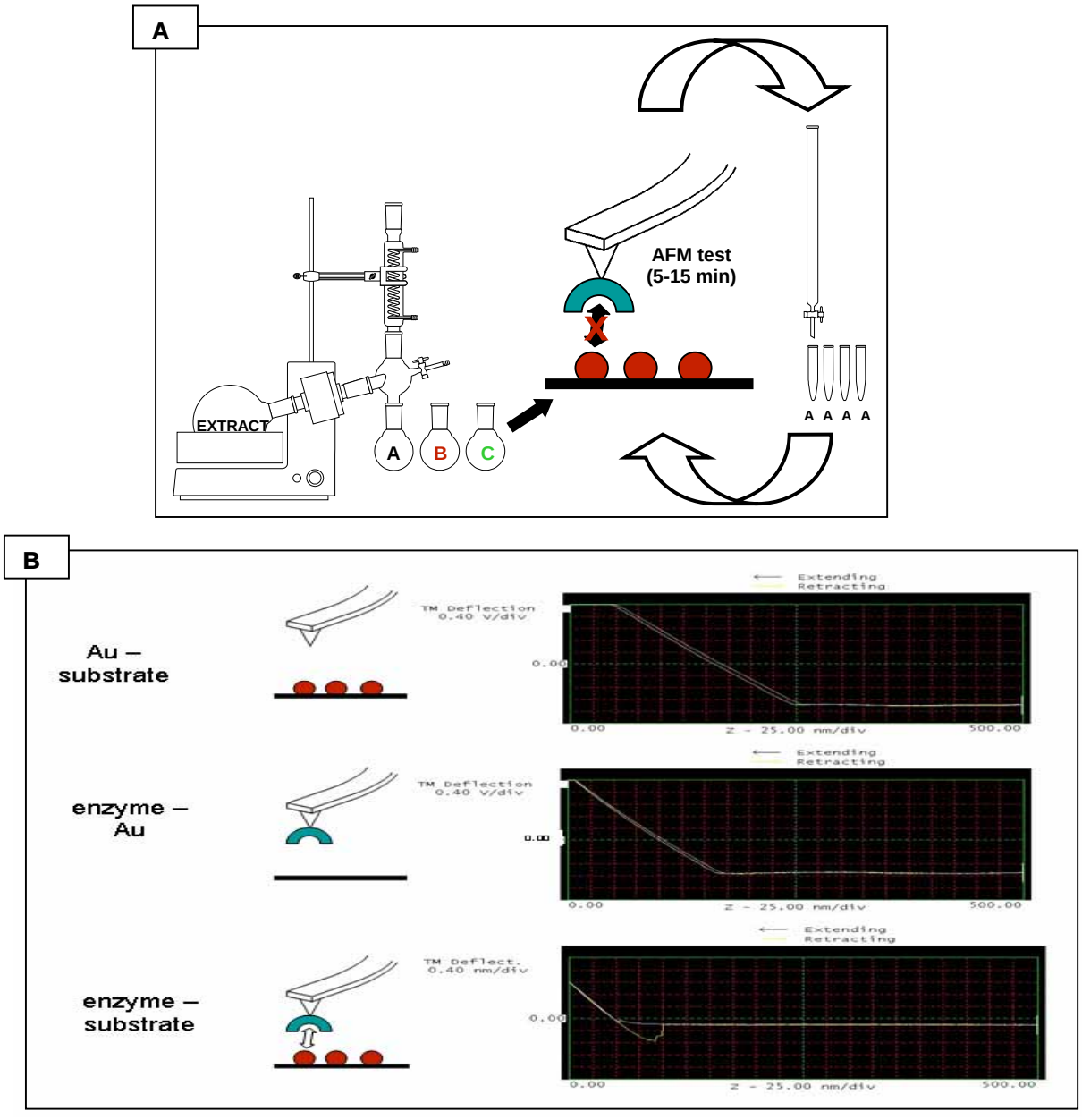

Figure 1. A: Schematic drawing of the experimental set-up. **B:** AFM force curves representing statistically significant interactions corresponding to the cases shown on the left.