Antitumor efficiency of the natural alkaloids complexed with C_{60} fullerene in Lewis lung carcinoma *in vitro* and *in vivo*

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Introduction

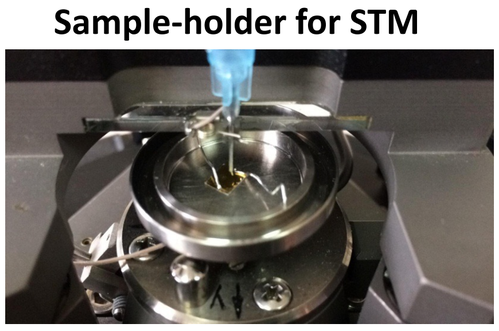
Medicines with small molecule sizes have unique advantages for medical usage due to non-immunogenic effect, variety of administration ways, simple absorption and preparation. Piperlongumine (PL) have multiple pharmacological properties and exhibit antitumor effect. However, its use is limited due to the low stability and bioavailability, as well as the need for use in high, toxic doses. The use of nanosize systems for the efficient delivery of natural alkaloids to tumor cells can help to solve this problem and increase their therapeutic efficacy.

We present the first study of C_{60} fullerene (C_{60}) - PL nanocomplex layers deposited from water solution. The results obtained using atomic force (AFM) and scanning tunneling microscopies (STM) provide the basis for further examination of the complexation between C_{60} and PL.

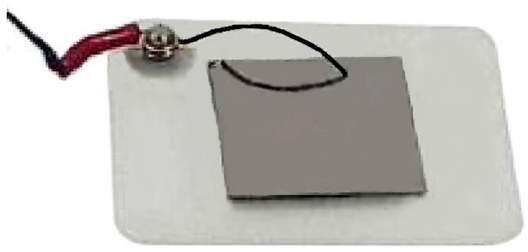
Also in this work we performed the structure modeling and accomplished decomposition of the net energy (ΔG_{total}) of complexation of C_{60} with PL molecule on the component energies originated from different physical factors, following the methodology of decomposition energy reported in [1]. The nanocomplexes of C_{60} with different number of PL molecules (from 1 to 8) were obtained by means of quantum-chemical method XTB2 [2] with implicitly specified water using Orca software.

Measurement procedures

Sample-holder for STM

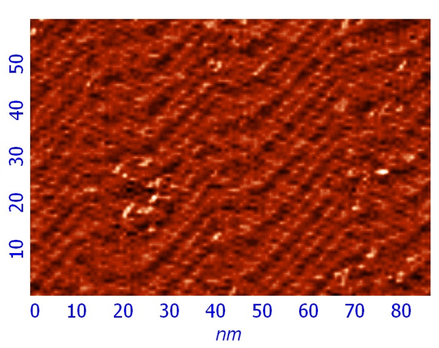


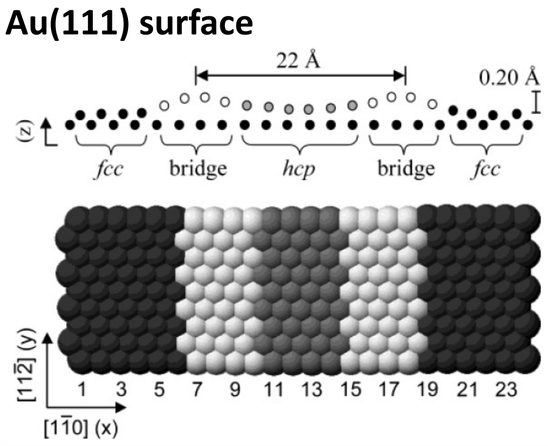
Sample-holder for AFM



Substrate

Reconstructed Au(111) surface





Results

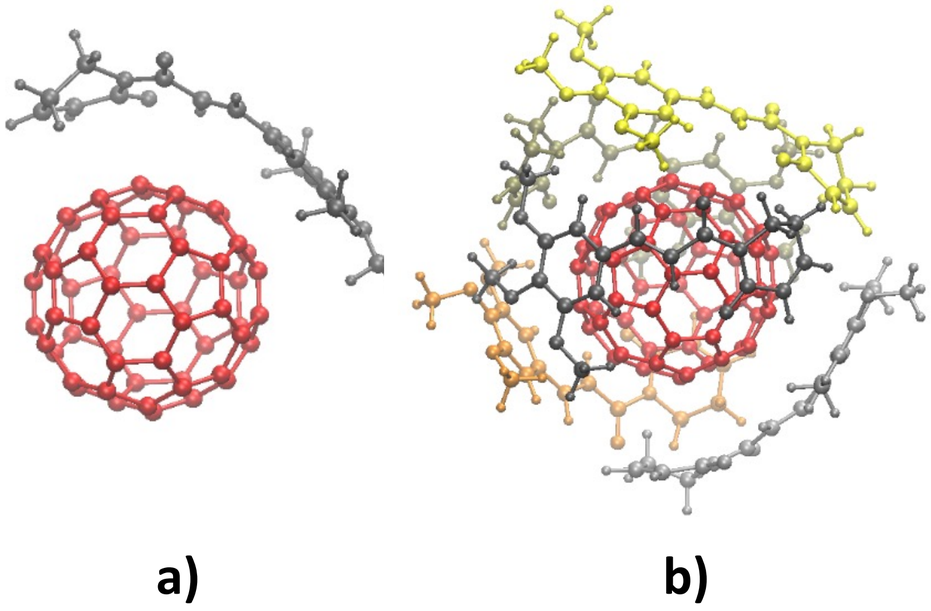


Fig. 1 The optimized structures of 1:1 (a) and 1:5 (b) C_{60}-PL nanocomplexes

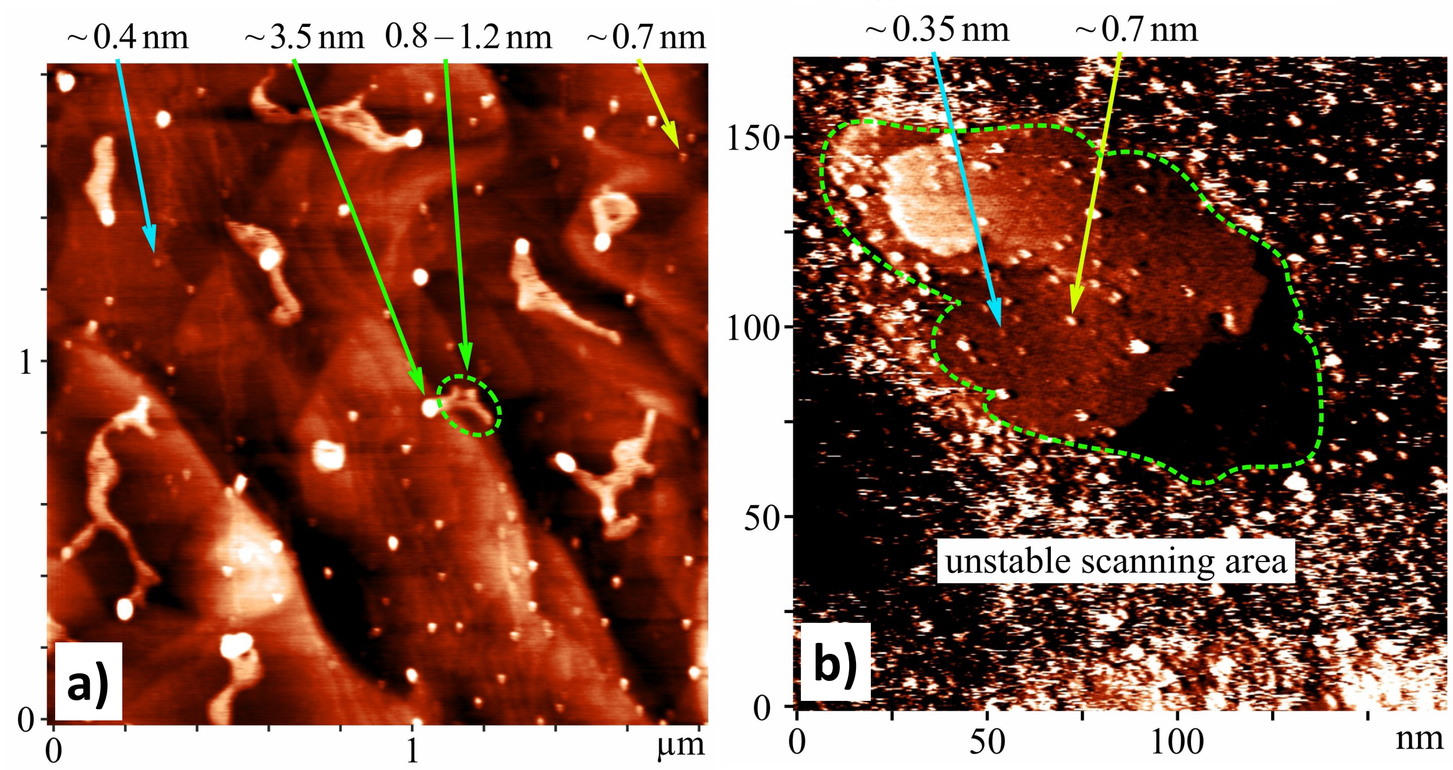


Fig. 2 AFM (a) and STM (b) image of thin films deposited from C_{60}+PL water solution on Au (111) surface. Scanning parameters for STM: I_t=47 pA, U_t=650 mV .

Using quantum-chemical method [2] we optimized nanocomplexes of C_{60} with different numbers n=1, 2,…, 8 of PL molecules, which enabled to estimate the maximal adsorption ability of one C_{60} against the binding of PL. It was found that the maximal number of PL molecules, which can interact with the C_{60} surface directly, equals to 5. In the case of nanocomplexes with more than 5 PL molecules we noted increased overlap (interaction) of PL chromophores resulting in stacking them one above other, which destabilizes the nanocomplex. Fig. 1 demonstrates the optimized structures of 1:1 and 1:5 C_{60}-PL nanocomplexes.

The AFM investigation of C_{60}-PL layers revealed the single objects ~ 0.4 nm and ~ 0.7 nm in height (Fig. 2a), which we identify as PL and C_{60} molecules, respectively. Additionally, we observed elongated conglomerates up to 1 µm in length, which were absent in the layers of pure C_{60} and PL. Therefore, it can be assumed that they are a mixture of C_{60} and PL. STM studies of a complex system were difficult in the so-called zones of instability due to disruptions of the tunneling current, self-excitation of the feedback circuit, and frequent contamination of the STM probe. In areas outside the instability zones, only point objects with heights characteristic of PL and C_{60} molecules separately were found (Fig. 2b). We explain this by the fact that the zones of instability correspond to the conglomerates observed in the AFM images. The PL layer inside the conglomerate is thick enough to disrupt the passage of the tunneling current.

The cytotoxicity of the studied C_{60}-PL agents follows the order: free PL<C_{60}-PL nanocomplex. C_{60}-PL nanocomplexes induce caspase 3/7 activation and suppress the migration activity of Lewis lung carcinoma (LLC) cells. The therapeutic potency of C_{60}-PL nanocomplexes is confirmed in a mouse model of LLC. This study indicates that complexation of natural alkaloid PL with C_{60} may be a novel therapeutic strategy against lung carcinoma.

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