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Report:

Chemotherapy is the routine treatment in first line defence especially in inoperable colorectal cancers, but there is still a need of development of delivery methods to improve drug distribution and bioavailability [2]. Among the drug delivery platforms, Virus-like Particles (VLPs) represent a promising strategy to efficiently transfer bioactive molecules to target tissues. VLPs consist of empty virus capsids unable to replicate but having the advantage of a highly evolved and efficient delivery strategy based on mimicking the natural behaviour of viruses. VLPs formed by Rotavirus capsid proteins display the intestinal tropism of Rotaviruses while lacking infectivity [3] and for this reason, represent ideal nanocarriers for the gut.

Our group has recently developed a fast and highly reproducible protocol to produce large amount of VP6 particles, based on the fusion of VP6 to small ubiquitin-like modifier (SUMO) [4]. SUMO enhances VP6 solubility and allows the possibility of obtaining the viral protein with a high degree of purity. The final step of protein production considers the cleavage of SUMO by a protease in order to obtain the purified VP6. However, SUMO could represent a convenient tag for proteins and drugs in chemotherapy since allows precise intranuclear localization [5]. For this reason, our aim is to investigate the stability of VP6 in presence of SUMO tag and the possible steric hindrance of SUMO in the process of assembly of VP6-SUMO particles. VP6 protein from human rotavirus A has been expressed in an E. Coli BL21 system following a recently developed protocol [4]. Purified protein at pH 7,4 in Tris HCl buffer self-assembles into trimers which are known to be the thermodynamically most stable form of VP6 under many conditions [3]. The theoretical value of the Hydrodynamic Radius of VP6 (PDB 1QHD). If the SUMO-VP6 construct is expressed, the trimeric form is lost and the chimeric protein self-assembles in a new thermodynamically stable structure.

Size properties of the SUMO-VP6 assembly have been studied by means of Dynamic Light Scattering (DLS) and compared to Small angle X Ray Scattering (SAXS) data acquired at ESRF. SUMO-VP6 at pH 7.4 in Tris-HCl Buffer has a R_H of 7.8 nm as is visible from the P(n) distribution in Fig.1A. The protein has a narrow size distribution indicating a high monodispersity. SAXS has been performed to retrieve radius of gyration (R_G) and Pair distribution function P(R) of the protein. The P(R) distribution is bell-shaped indicating a compact globular structure with a R_G of 7.9 nm, consistent with value obtained with DLS (Fig.1B). This value has been confirmed also with Guinier analysis. The theoretical R_H value from the 3D structure is 3.3 nm, much smaller than the experimental R_H of 7.9 nm, hence we can conclude that several subunits self-assemble to form a single carrier. We are currently analyzing tridimensional properties of the assembly with DAMMIN software.



Figure 1 (A) P(n) distribution of VP6-Sumo oligomer obtained with DLS (B) P(r) distribution of VP6-Sumo oligomer from SAXS measurement. A representative SAXS curve is shown in the inset in blue. The fit performed by GNOM is in red in the inset.

We tested the uptaxe of the carriers on Colon Cancer Cells and analyzed the localization after endocytosis of the carriers in a recent paper [6].

The data on this report are still preliminary and we are analyzing the behaviour of VP6-SUMO assemblies in different pH and ionic strenght conditions.

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