Revealing Binding Position of Aplyronine A as a Protein-Protein Interaction Inducer

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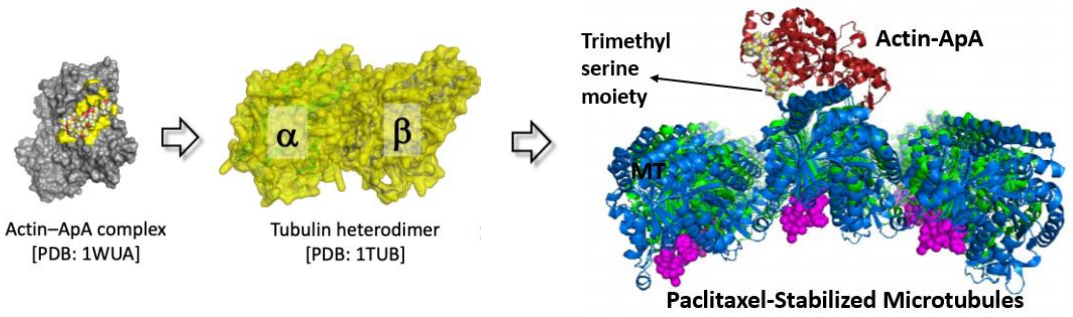
Aplyronine A (ApA) is an antitumor macrolide that stabilizes the protein-protein interaction between actin and tubulin[1]. This study aims to evaluate the binding position of actin–aplyronine A (ApA) complex on tubulin and microtubule (MT) lattice. 3D structure of actin-ApA (1WUA) and tubulin (1TUB) were retrieved from PDBJ.org[2,3]. Protein-protein docking technique in Molecular Operating Environment (MOE.2019.1) was employed for determining the binding position of actin–ApA complex on tubulin. These results showed that actin–ApA complex had 10 possible binding models on tubulin heterodimers with the best affinity of –81.7 kcal/mol. The protein-protein interaction was clearly mediated by ApA and the stable ternary complex was supported by hydrogen bonds and hydrophobic interactions. Predicted ternary complex revealed that actin–ApA complex preferred the Helix-11 (H11) and Helix-12 (H12) for its binding position on tubulin.

Furthermore, reaction of actin–ApA with paclitaxel-stabilized MT was conducted at 37 °C for 1 hour, and their interactions were evaluated after multi-ultracentrifugation strategy (58.000 rpm and 120.000 rpm) using SDS-PAGE. Negative stain in electron microscopy showed that actin–ApA complex directly broke paclitaxel-stabilized MT. Most of the tubulin became a ternary complex, and some short fragments were broken when interacted with actin–ApA complex. This finding will be further validated by 3D-cryo-EM.



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