Probing the Small-Molecule Inhibition of an Anticancer Therapeutic Protein-Protein Interaction Using a Solid-state Nanopore

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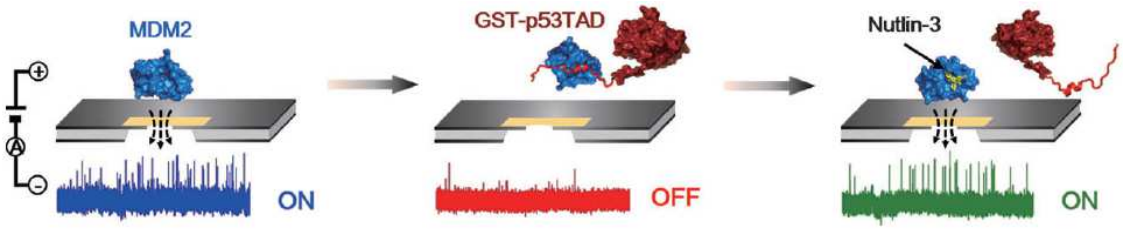
Nanopore sensing is an emerging technology for the single-molecule-based detection of various biomolecules. In this study, we probed the anticancer therapeutic p53 transactivation domain (p53TAD)/MDM2 interaction and its inhibition with a small-molecule MDM2 antagonist, Nutlin-3, using low-noise solid-state nanopores. Although the translocation of positively charged MDM2 through a nanopore was detected at the applied negative voltage, this MDM2 translocation was almost completely blocked upon formation of the MDM2/GST-p53TAD complex owing to charge conversion. In combination with NMR data, the nanopore measurements showed that the addition of Nutlin-3 rescued MDM2 translocation, indicating that Nutlin-3 disrupted the MDM2/GSTp53TAD complex, thereby releasing MDM2. Negative control of ABT-737, which is an inhibitor of Bcl-2 family proteins, could not recover the translocation of MDM2, confirming that Nutlin-3 specifically blocked the interaction between MDM2 and GST-p53TAD. Taken together, our results reveal that solid-state nanopores can be a valuable platform for the ultrasensitive, picomole-scale screening of small-molecule drugs against protein–protein interaction (PPI) targets.



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