Use of molecular dynamics to decipher the binding of salicylic acid to proteins. Example of Arabidopsis thaliana Chloroplastic GAPDH-A1

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Abstract: Salicylic acid (SA) has an essential role in the responses of plants to pathogens. SA initiates defense signaling cascades via binding to proteins. NPR1 is a transcriptional *co*-activator and is a key target of SA binding. Many other proteins have been shown to bind SA. Amongst these proteins are important enzymes of primary metabolism. Here, we detail that the A1 isomer of chloroplast glyceraldehyde 3-phosphate dehydrogenase (GAPA1) from *Arabidopsis thaliana* binds SA, as shown in surface plasmon resonance experiments. Besides, we show that SA inhibits its GAPDH activity in *vitro*. To gain some insight into the underlying molecular interactions and binding mechanism, we combined in silico molecular docking experiments and molecular dynamics simulations on the free protein and protein–ligand complex. The molecular docking analysis yielded to the identification of two putative binding pockets for SA. A simulation in water of the complex between SA and the protein allowed us to determine that only one pocket—a surface cavity around Asn35—would efficiently bind SA in the presence of solvent. The importance of this is further supported through experimental biochemical assays. Indeed, mutating GAPA1 Asn35 into Gly or Arg81 into Leu strongly diminished the ability of the enzyme to bind SA. The very same cavity is responsible for the $NADP^+$ binding to GAPA1. NADH inhibited, in a dose-response manner, the binding of SA to GAPA1, validating our data. The use of the methodology to study SA binding to other proteins will be discussed at the end of the talk.

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