

Supporting Information

Jiang *et al.* 10.1073/pnas.0810962106

SI Text

Molecular Modeling. Molecular modeling was based on the crystal structure (2.9 Å resolution) of COX-1 complexed with linoleic acid (PDB ID code 1IGZ) (1) due to similar length as 13'-carboxychromanol. 13'-carboxychromanol, 9'-carboxychromanol, and AA were docked into the binding pocket using AutoDock 4 (2). The side chains of the most flexible residues within the binding pocket were identified based on a Concord (3) simulation and computed b-factors from a molecular dynamics simulation of the apo protein. The side chains of these residues, Asn-375 and Ser-530, were treated as flexible throughout the docking simulation. To identify probable binding modes, 50 genetic algorithm runs were performed with a population size of 300, maximum number of generations of 27,000, and 50 million energy evaluations. The best solutions were automatically clustered based on geometric similarity.

To investigate the stability of the proposed docking configuration and to reliably predict the relative binding affinities of 13'-, 9'-carboxychromanol, and AA, molecular dynamic (MD) simulations were ran for all docking complexes using GRO-MACS (4). The Amber 2003 force field was used for the protein

and the gaff force field for the ligand. Periodic boundary conditions were applied using a truncated octahedron with a minimum protein-face distance of 10 Å ($\approx 74,000$ atoms in total). A timestep of 2 fs was chosen using the LINCS algorithm for constraining bonds involving hydrogen atoms. Each initial docked structure was equilibrated using 5,000 steps of steepest-descent minimization, 250 ps MD simulation for equilibration of the water molecules keeping the protein restrained, and 1.25 ns MD simulation without any restraints.

Based on a subsequent 500 ps MD simulation for data collection, molecular mechanics Poisson-Boltzmann surface area (MM/PBSA) method (5) and linear interaction energy (LIE) analysis (6) were applied. Both methods failed to reproduce the X-ray structure of COX-1-bound AA as the energetically lowest configuration. In MM/PBSA, high standard deviations (on the order of 10–15 kcal/mol) in the computed entropy contribution prevented a clear separation between different binding modes. In LIE, the dominating electrostatic interactions of the charged AA with protein and solvent are vastly fluctuating and dependent on the solvent configuration. Standard deviations were 10–20 kcal/mol.

1. Malkowski MG, *et al.* (2001) Structure of eicosapentaenoic and linoleic acids in the cyclooxygenase site of prostaglandin endoperoxide H synthase-1. *J Biol Chem* 276:37547–37555.
2. Morris GM, *et al.* (1998) Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. *J Comput Chem* 19:1639–1662.
3. de Groot BL, *et al.* (1997) Prediction of protein conformational freedom from distance constraints. *Proteins* 29:240–251.
4. van der Spoel D, *et al.* (2005) GROMACS: Fast, flexible and free. *J Comp Chem* 26:1701–1718.
5. Kollman PA, *et al.* (2000) Calculating structures and free energies of complex molecules: Combining molecular mechanics and continuum models. *Acc Chem Res* 33:889–897.
6. Aqvist J (1996) Calculation of absolute binding free energies for charged ligands and effects of long-range electrostatic interactions. *J Comput Chem* 17:1587–1597.

