Warshel, A., & Levitt, M. (1976). Theoretical Studies of Enzymic Reactions - Dielectric, Electrostatic and Steric Stabilization of the Carbonium Ion in the Reaction of Lysozyme. *Journal of Molecular Biology, 103*(2), 227-249.

***Background***

* *Enzymes* are large biological molecules responsible for thousands of metabolic processes that sustain life, including food digestion and DNA synthesis.
  + They are highly selective catalysts that greatly accelerate the *rate* and *specificity* of metabolic reactions.
  + Most enzymes are *proteins* and they adopt a specific three-dimensional structure.
  + Enzymes may employ organic or inorganic *cofactors* to assist in catalysis.
* *Cofactors* are non-protein compounds that are bound to a protein and are required for the protein’s biological activity.
  + “Helper molecules” that assist in biochemical transformations.
  + Organic cofactors include biotin, flavin and heme. They can be further classified as:
    - *Coenzymes* 🡪 loosely bound cofactors that refer to the functional properties of a protein.
    - *Prosthetic groups* 🡪 tightly bound (covalent) cofactors that refer to a structural property.
  + Inorganic cofactors include:
    - *Metal ions* 🡪 metalloproteins
    - *Iron-sulfur clusters* 🡪 sulphide-linked di-, tri- and tetra-iron centres in variable oxidation states
* *Substrates* are molecules upon which an enzyme acts.
  + For a single substrate, an *enzyme-substrate complex* forms when the substrate bonds with the enzyme active site.
  + *Active sites* 🡪 small areas of an enzyme where substrates bind and undergo a chemical reaction.
  + *Binding* 🡪 usually enzymes have only one active site which fits with one specific type of substrate.
* Enzymes can be *denatured* at high temperatures or extreme pH values.
  + This causes the active site to change shape and the substrate molecules to no longer fit.
  + The *efficiency* of a reaction is believed to increase when there is a tighter fit between the active site and the substrate.
* *Solvation* is the interaction of molecules of a *solvent* with ions of a *solute* which leads to the stabilisation of the solute species in the solution.
  + Ions spread out and dissolve in a solvent and become surrounded by solvent molecules.
* *Dielectrics* are electrical insulators that can be polarized by an applied electric field.
  + Electrical charges do not flow through dielectric material when it is placed in an electric field.
  + Instead, there is a slight shift in average equilibrium positions causing dielectric polarization.
* *Solvation energy* resulting from polarization is quite considerable and must be accounted for when studying enzyme reactions. This allows:
  + Acidic groups to become ionized.
  + Charge distribution on substrate to be reasonable.
* *Bond cleavage* is the process of breaking down large molecules by splitting their internal bonds.
* *Charge distribution (density)* in quantum mechanics is summarised by the following equations.
  + 🡪 related to wavefunction
    - =charge of the particle
    - = probability density function 🡪 probability per unit volume of a particle located at
  + 🡪 when wavefunction is normalized
    - = the average charge in the region
    - = the integration measure over 3-D space

***Outline***

* *Aim:* To develop a theoretical method for studying the reactions of enzymes, with particular emphasis on the factors that affect reaction pathways.
* *Scope:*
  + The system includes the entire enzyme-substrate (E-S) complex and its solvent (water).
  + The reaction examined is the cleavage of theglycosidic bondof *lysozyme*, focusing on the factors that affect the stability of the carbocation intermediate formed.
  + Classical approaches use *empirical energy functions* to study the conformationof E-S complexes 🡪 based on bond stretching, angle-bending, twisting and non-bonded interactions.
  + Quantum mechanical (QM) approaches can study the mechanism and energetics of enzyme reactions, although it has several limitations:
    - Model is oversimplified, including only a small fraction of atoms involved in the reaction.
    - Reactions are treated within an isolated system (vacuum) and are not able to account for “dielectric effects” (polarizability of atoms) which affect the energy contributions from electrostatic interactions.
* *Method:*
  + The complete enzyme-substrate-solvent system examined includes all the energy factors that might contribute to the reaction mechanism, including:
    - *QM energy factors:*
      * + Bond cleavage (rearrangement of bonds)
        + Charge redistribution (density) of the substrate
    - *Classical energy factors:*
      * + Steric interactions (substrate strain on binding to the enzyme)
        + Electrostatic interactions (between substrate and enzyme)
    - *Dielectric effects*:
      * + Polarization of the enzyme atoms
        + Dipole orientation of water molecules
* *Model:*
  + The proposed *hybrid classical/QM* model evaluates:
    - *Energy* and *charge distribution* of the atoms that directly participate in the reaction (QM methods).
    - *Potential energy surface* of the rest of the system, which includes steric and electrostatic interactions (classical methods).
    - *Dielectric effects* from induced dipoles and polarization (QM/classical).
* *Analysis:* 
  + *Potential energy partition*
    - The model partitions the potential energy surface into classical and quantum mechanical components, as well as a “coupling” term.
    - The combined coupling term accounts for:
      * Induced dipoles in the classical region
      * Electrostatic and van der Waals interactions between atoms in the quantum and classical regions.

* + - obtained by an extended form of *QCFF/PI* (quantum-mechanical consistent force field method for pi-electron systems) that includes all valence electrons 🡪 referred to as *QCFF/ALL*

* + *Induced dipoles and the effective dielectric*
  + *Substrate conformational changes*
* *Results:*
  + *Steric effects in the ground state*
  + *Equilibrium conformation of the carbonium ion*
  + *Charge stabilization and dielectric effect*
* *Conclusions:*
  + By incorporating the polarizability of atoms into the hybrid model through dielectric effects, a more accurate description of electrostatic interactions and its energy contributions is achieved.
  + Electrostatic interaction is a major factor in the reaction rate leading to carbocation intermediate formation.
  + Steric strain was found to be of minor importance.