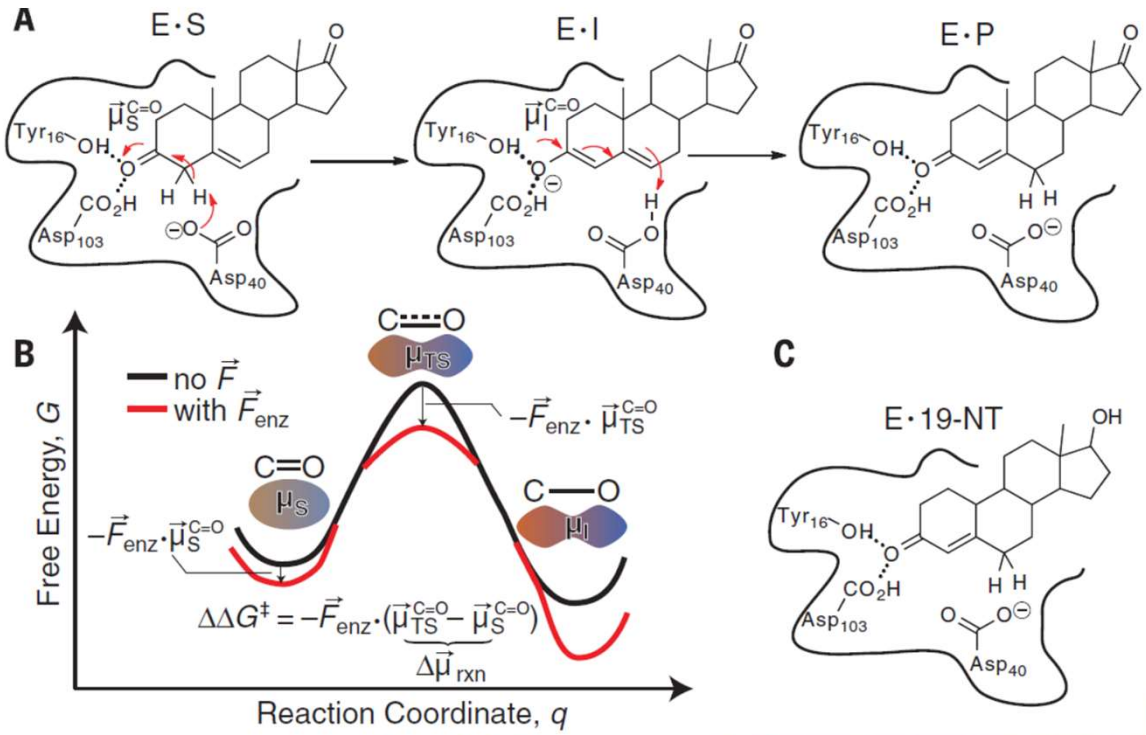
But these questions are still controversial!

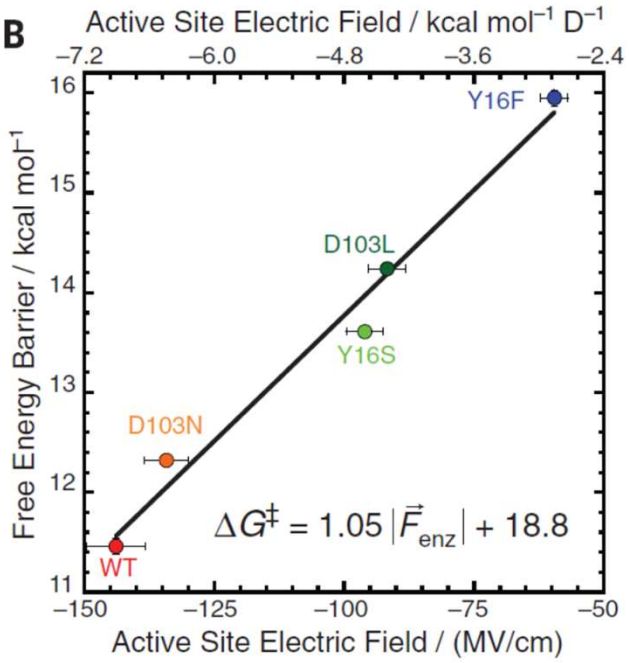
BIOPHYSICS

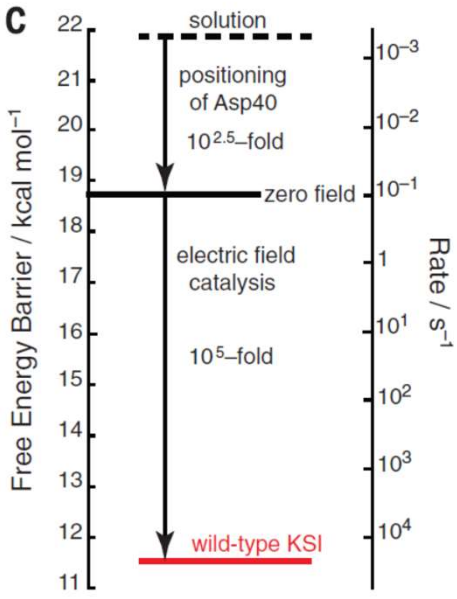
Extreme electric fields power catalysis in the active site of ketosteroid isomerase

Stephen D. Fried,* Sayan Bagchi,† Steven G. Boxer‡

Enzymes use protein architecture to impose specific electrostatic fields onto their bound substrates, but the magnitude and catalytic effect of these electric fields have proven difficult to quantify with standard experimental approaches. Using vibrational Stark effect spectroscopy, we found that the active site of the enzyme ketosteroid isomerase (KSI) exerts an extremely large electric field onto the C=O chemical bond that undergoes a charge rearrangement in KSI's rate-determining step. Moreover, we found that the magnitude of the electric field exerted by the active site strongly correlates with the enzyme's catalytic rate enhancement, enabling us to quantify the fraction of the catalytic effect that is electrostatic in origin. The measurements described here may help explain the role of electrostatics in many other enzymes and biomolecular systems.







Nils Walter: Chem 451

Resulting in a scientific back-and-forth

TECHNICAL COMMENT

BIOPHYSICS

Comment on "Extreme electric fields power catalysis in the active site of ketosteroid isomerase"

Aditya Natarajan,[1] Filip Yabukarski,[1] Vandana Lamba,[1] Jason P. Schwans,[2] Fanny Sunden,[1] Daniel Herschlag[1]*

Fried *et al.* (Reports, 19 December 2014, p. 1510) demonstrated a strong correlation between reaction rate and the carbonyl stretching frequency of a product analog bound to ketosteroid isomerase oxyanion hole mutants and concluded that the active-site electric field provides 70% of catalysis. Alternative comparisons suggest a smaller contribution, relative to the corresponding solution reaction, and highlight the importance of atomic-level descriptions.

TECHNICAL RESPONSE

BIOPHYSICS

Response to Comments on "Extreme electric fields power catalysis in the active site of ketosteroid isomerase"

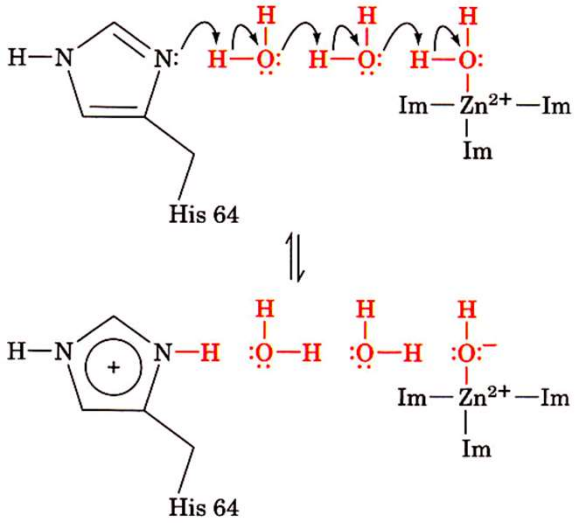
Stephen D. Fried* and Steven G. Boxer†

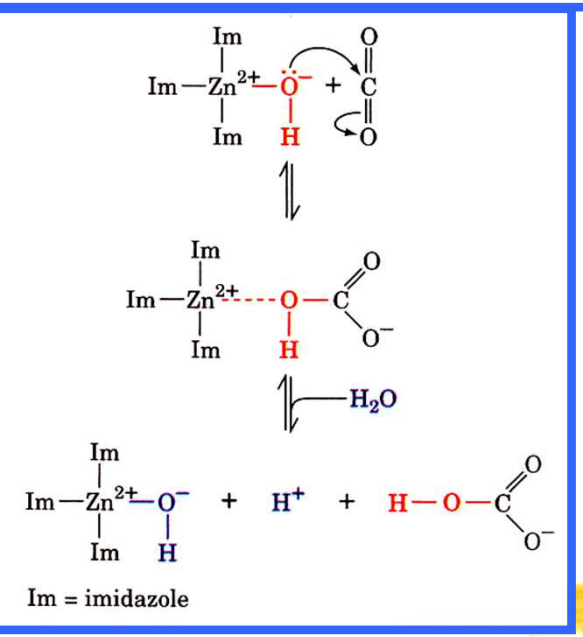
Natarajan *et al.* and Chen and Savidge comment that comparing the electric field in ketosteroid isomerase's (KSI's) active site to zero overestimates the catalytic effect of KSI's electric field because the reference reaction occurs in water, which itself exerts a sizable electrostatic field. To compensate, Natarajan *et al.* argue that additional catalytic weight arises from positioning of the general base, whereas Chen and Savidge propose a separate contribution from desolvation of the general base. We note that the former claim is not well supported by published results, and the latter claim is intriguing but lacks experimental basis. We also take the opportunity to clarify some of the more conceptually subtle aspects of electrostatic catalysis.

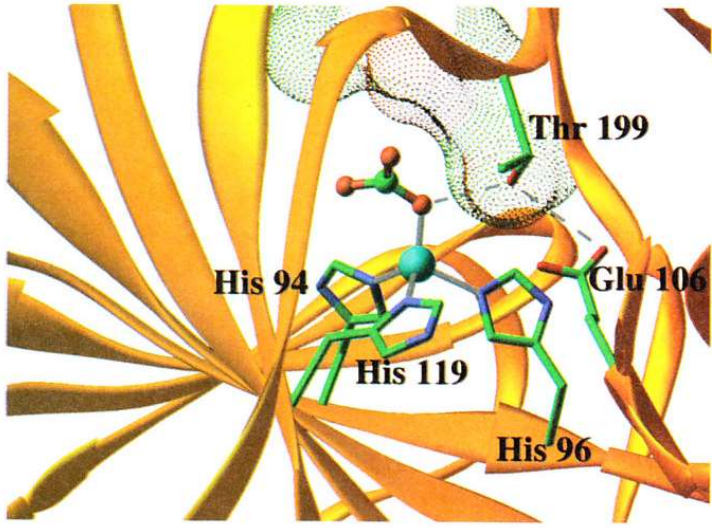
both the functional groups that are present and their positioning. This positioning arises from the folding of the protein, using favorable folding energy to orient and restrict the conformational mobility of these groups, and from binding of the substrate, in a pocket also formed due to folding of the protein (*6*–*8*). Turning to the substrate, if there were no pocket or if the substrate were sterically restricted from approaching the oxyanion hole, then there would be less or no catalysis; if the substrate were bound but positioned such that its carbonyl group faced away from the oxyanion hole, then the oxyanion hole and its surroundings would not contribute to catalysis. In summary, electrostatic catalysis, to be effective, requires positioning—proper positioning of the substrate via binding interactions into a pocket that is created via protein folding as well as proper positioning of enzymatic groups, again via protein folding and substrate binding, to make favorable electrostatic interactions in the reaction's transition state. Thus, catalytic contributions from electrostatics and positioning appear to be inextricably linked. Understanding this linkage, and catalysis, will likely require descriptions that extend beyond measures of apparent electric fields to atomic-level descriptions and models, including the multiple states present in the ensemble of an enzyme-substrate

Metal ion catalysis: Substitutes for protons

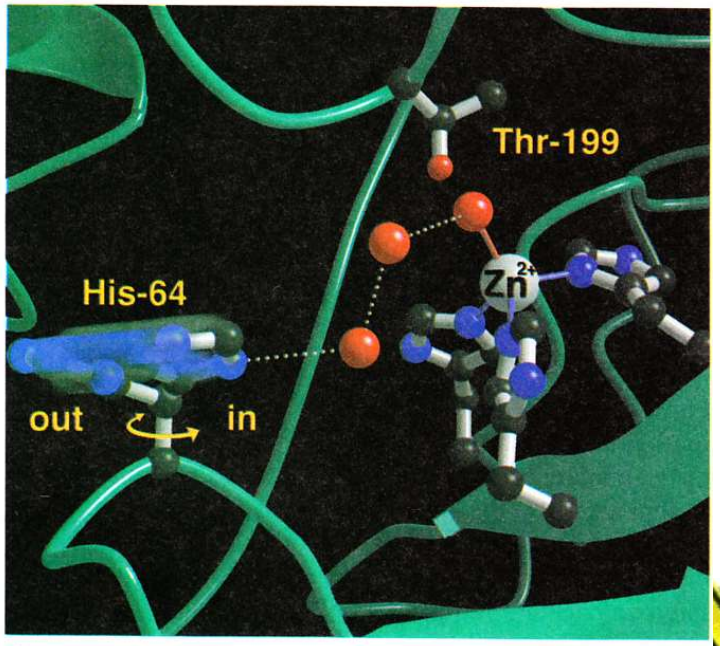
Example: Carbonic anhydrase







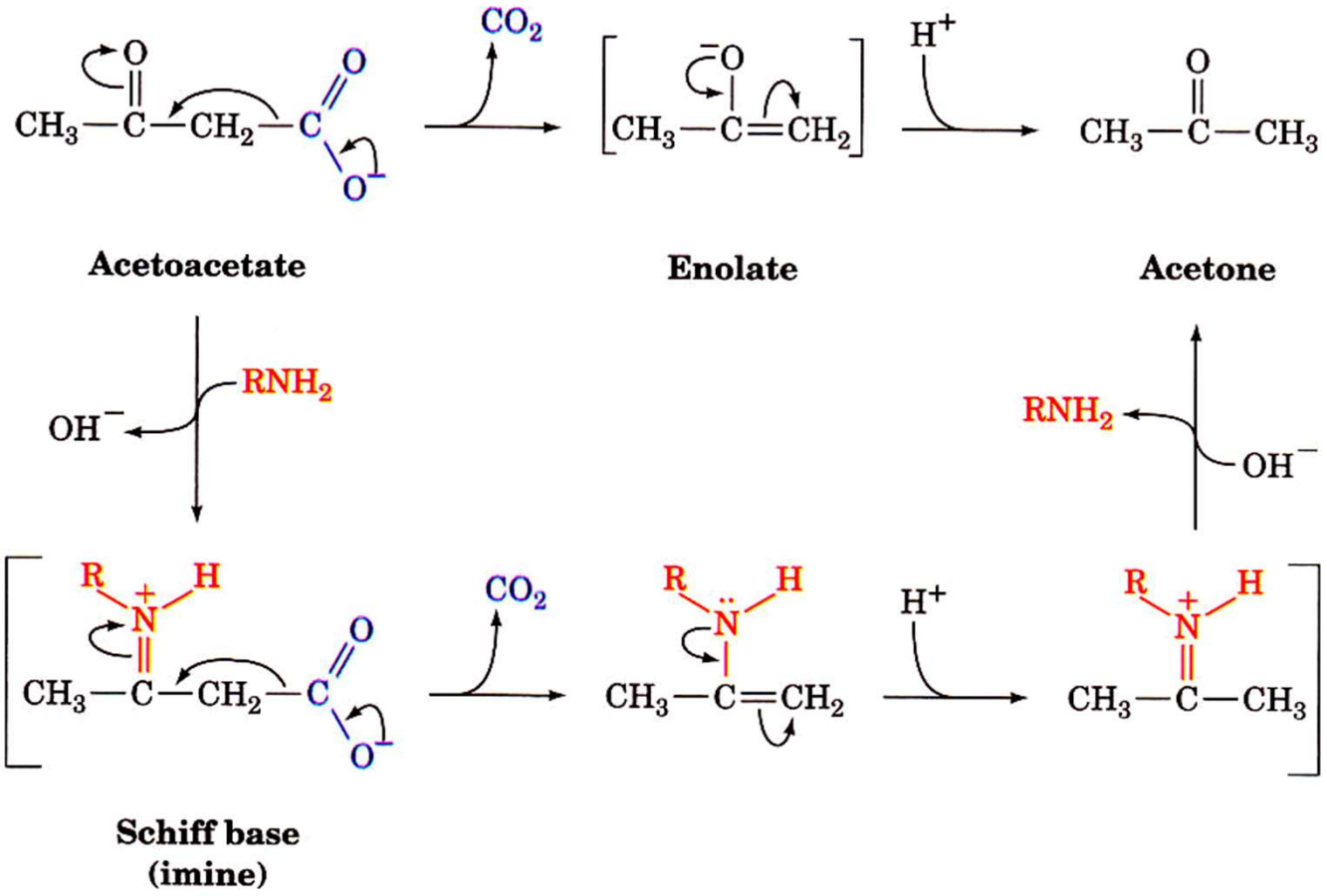
(a)



(b)

Covalent catalysis

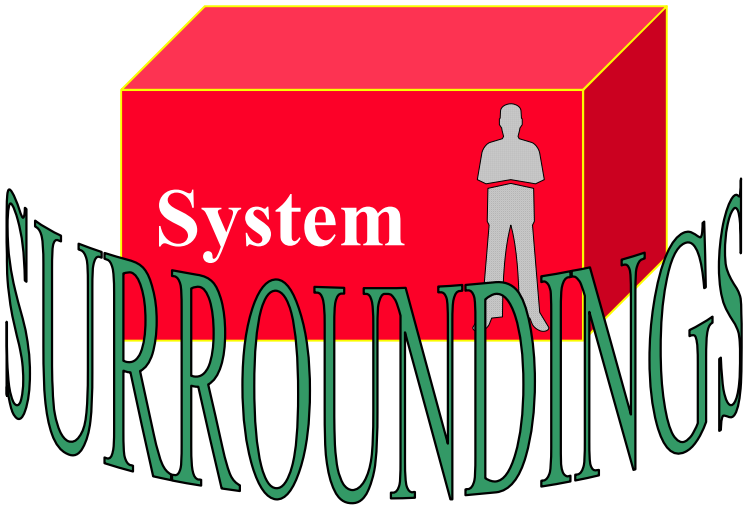
Example: Formation of Schiff base enhances electrophilicity



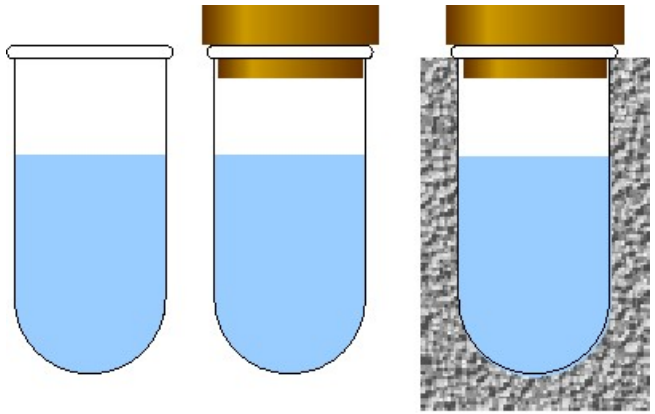
Important: Covalent bond must be formed with a strong nucleophile AND good leaving group

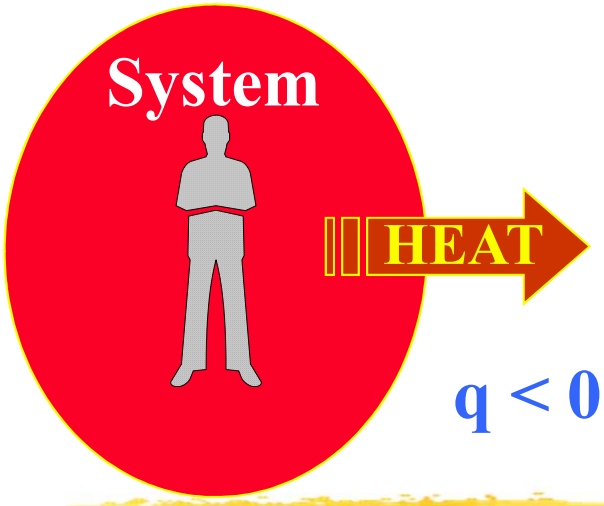
Thermodynamics: The First Law

Voet & Voet, Chapter 3; Atkins, Chapter 2



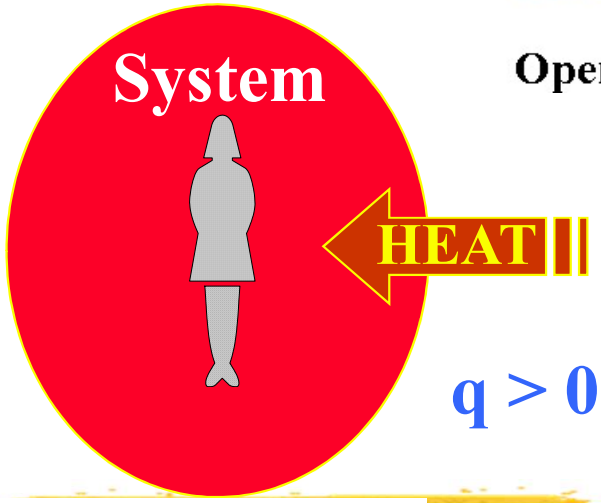
- Open System: Mass, heat, energy flow freely
- Closed System: Heat, energy flow freely
- Isolated System: No mass, heat, or energy flow





$q < 0$

Exothermic



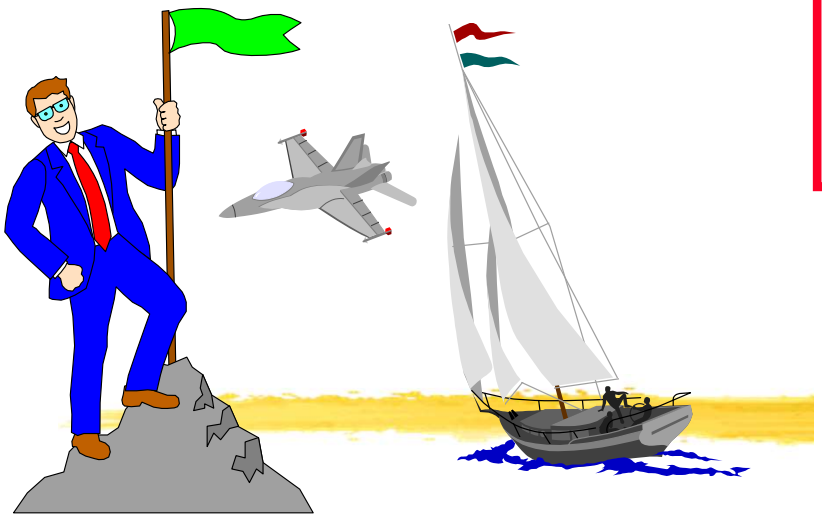
$q > 0$

Endothermic

The First Law of Thermodynamics: Internal Energy is Conserved

- The change in internal energy (ΔU) of a closed system is equal to the sum of the heat (q) added to it and the work (w) done upon it
- The internal energy of an isolated system is constant

$\Delta U = q + w$	For a Closed System
$\Delta U = 0$	For an Isolated System



Internal energy U is a state function
$\Rightarrow$ Quantity is independent of path

Volume, Temperature, Pressure, and Quantity are other examples of state functions

Internal Energy and Enthalpy

Enthalpy definition: $H = U + pV$

Most convenient for processes at constant pressure:

- Cooking dinner
- Drying the laundry
- Digesting dinner
- Synthesizing a compound in lab

At constant pressure, if only pV work is done:

$$\Delta U = q + w = q_p - \int_{V_1}^{V_2} p\, dV$$

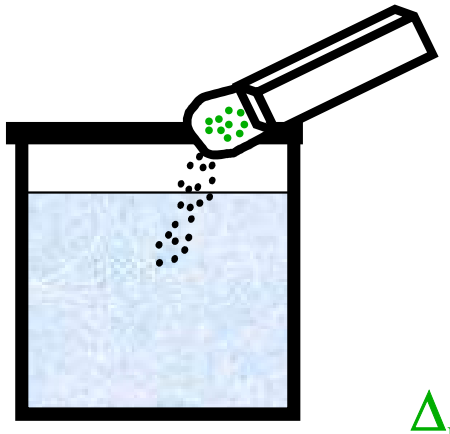
$$= q_p - p\int_{V_1}^{V_2} dV = q_p - p(V_2 - V_1) = q_p - p\Delta V$$

p independent of V

$$\Delta H = \Delta U + p\Delta V = q_p$$

Enthalpy is the heat transferred in a process at constant pressure (assuming only pV work)

But there's more: Disorder



-411.15 kJ/mol -240.12 kJ/mol -167.16 kJ/mol

$$NaCl_{(s)} \rightarrow Na^{+}_{(aq)} + Cl^{-}_{(aq)}$$

Hess: $\Delta_r H^{\ominus} = \sum \nu \Delta_f H^{\ominus}(\text{Prod.}) - \sum \nu \Delta_f H^{\ominus}(\text{React.})$

$\Delta_r H = [-240.12 - 167.16 - (-411.15)]$ kJ/mol $= +3.87$ kJ/mol

This is an endothermic reaction - but clearly spontaneous, as was the endothermic reaction demonstrated in Chem 130:

$$2\ NH_4SCN_{(s)} + Ba(OH)_2 \bullet 8H_2O_{(s)} \rightarrow 2NH_{3\ (g)} + Ba(SCN)_{2\ (s)} + 10\ H_2O_{(l)}$$

The reverse reactions are not spontaneous

Qualitatively: "Nature Prefers Disorder"

$NaCl_{(s)}$ "Ordered" $\rightarrow$ $Na^{+}_{(aq)} + Cl^{-}_{(aq)}$ "Disordered"