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The Ascorbate Radical as a Universal Group Transfer Catalyst in Inflamed Tissue

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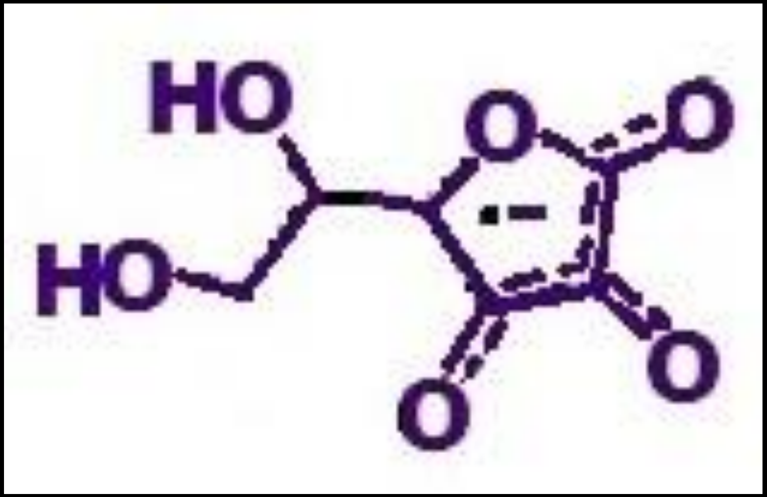
OVERVIEW: Central Hypothesis

The Ascorbate radical is a Biological Group Transfer Catalyst in Inflamed Tissue

- Sulfuryl group transfer (transsulfurylation)
- Phosphoryl group transfer (transphosphorylation)
- Nitrosyl group transfer (transnitrosylation)
- Acyl group transfer (transacylation)
- Glycosyl group transfer (transglycosylation)

CONCEPTUAL OVERVIEW:



- <u>Sulfuryl</u> group transfer
- <u>Phosphoryl</u> group transfer
- <u>Nitrosyl</u> group transfer
- <u>Acyl</u> group transfer
- <u>Glycosyl</u> group transfer

DEFINITION:

CATALYST / CATALYSIS / AUTOCATALYSIS -

“A substance that increases the rate of a reaction without modifying the overall standard Gibbs energy change in the reaction; the process is called catalysis. The catalyst is both a reactant and product of the reaction.”

“Catalysis can be classified as homogeneous catalysis, in which only one phase is involved, and heterogeneous catalysis, in which the reaction occurs at or near an interface between phases. Catalysis brought about by one of the products of a reaction is called autocatalysis. Catalysis brought about by a group on a reactant molecule itself is called intramolecular catalysis.”

Source:
PAC, 1996, 68, 149

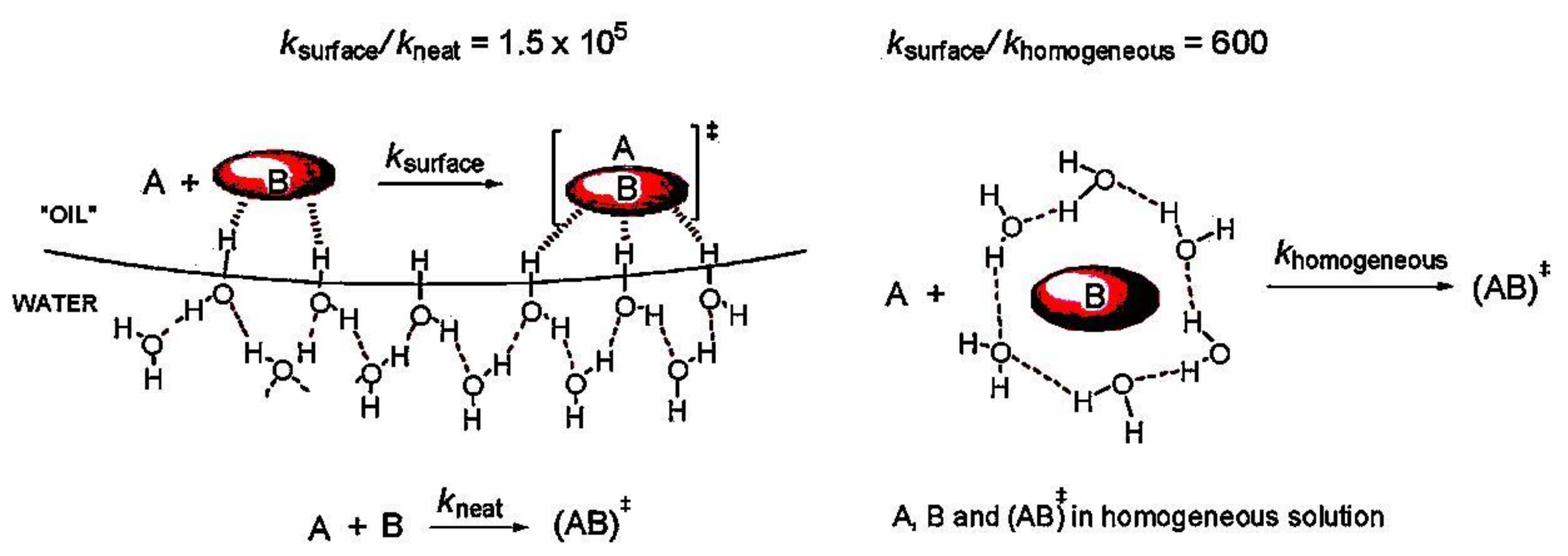
SOLVENT EFFECTS

<u>DEFINITION</u>: Heterogeneous Phase Catalysis

- Marcus' "on water" heterogeneous phase <u>catalysis</u>

- Exogenous Interfacial Water Stress (EIWS) Theory of Inflammation and Disease

Marcus' "On-Water" Heterogeneous Phase Catalysis



SOURCE:

Jung, Y. and Marcus, R.A. (2007). On the Theory of Organic Catalysis "on Water". *Journal of the American Chemical Society,* **129,** 5492-5502.

THE INITIAL COMMON PATHWAY

Exogenous Interfacial Water Stress (EIWS)

Davidson, Robert M.; Seneff, Stephanie. 2012.
Entropy 14, no. 8: 1399-1442.

Inflammation

Davidson, R.M.; Lauritzen, A.; Seneff, S. 2013.
Entropy **2013**, *15*, 3822-3876.

Disease

Five routes to the Ascorbate radical

- Radical-scavenger route

  $\mathrm{AscH^{-}} + \mathrm{R^{\bullet}} \leftrightarrows \mathrm{Asc^{\bullet -}} + \mathrm{RH}$

- Dismutation route

  $2\,\mathrm{Asc^{\bullet -}} + \mathrm{H^{+}} \leftrightarrows \mathrm{AscH^{-}} + \mathrm{DHA}$

- Hydated electron route

  $\mathrm{DHA} + \mathrm{e\,aq^{-}} \leftrightarrows \mathrm{Asc^{\bullet -}}$

- Transition metal-catalyzed route

  $\mathrm{AscH^{-}} + \mathrm{Cu^{2+}} \leftrightarrows \mathrm{Asc^{\bullet -}} + \mathrm{Cu^{1+}} + \mathrm{H^{+}}$

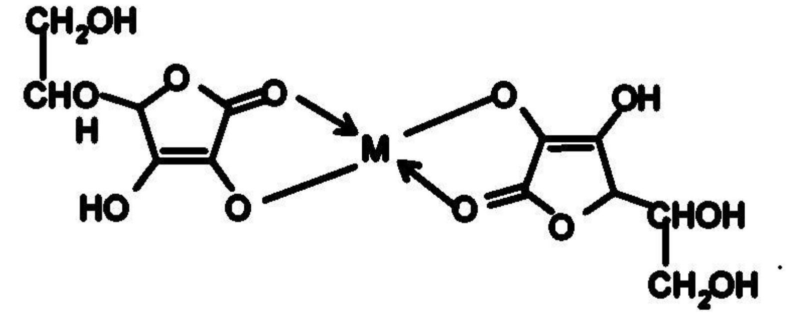
- “slow and continuous” radical-scavenger route

  $\mathrm{Asc\ 2\text{-}S} + \mathrm{Asc^{\bullet -}} + \mathrm{H^{+}} \leftrightarrows [\mathrm{Asc\ 2\text{-}S}]^{\bullet -} + \mathrm{AscH^{-}}$

Copper-Ascorbate Complex

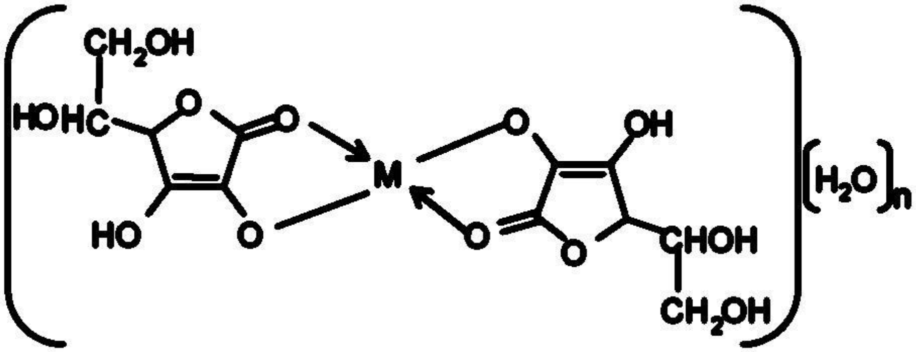
<u>Source</u>:

L. Amudat, Ph.D.
Dissertation, 2010



M = Cu (II), Zn (II)

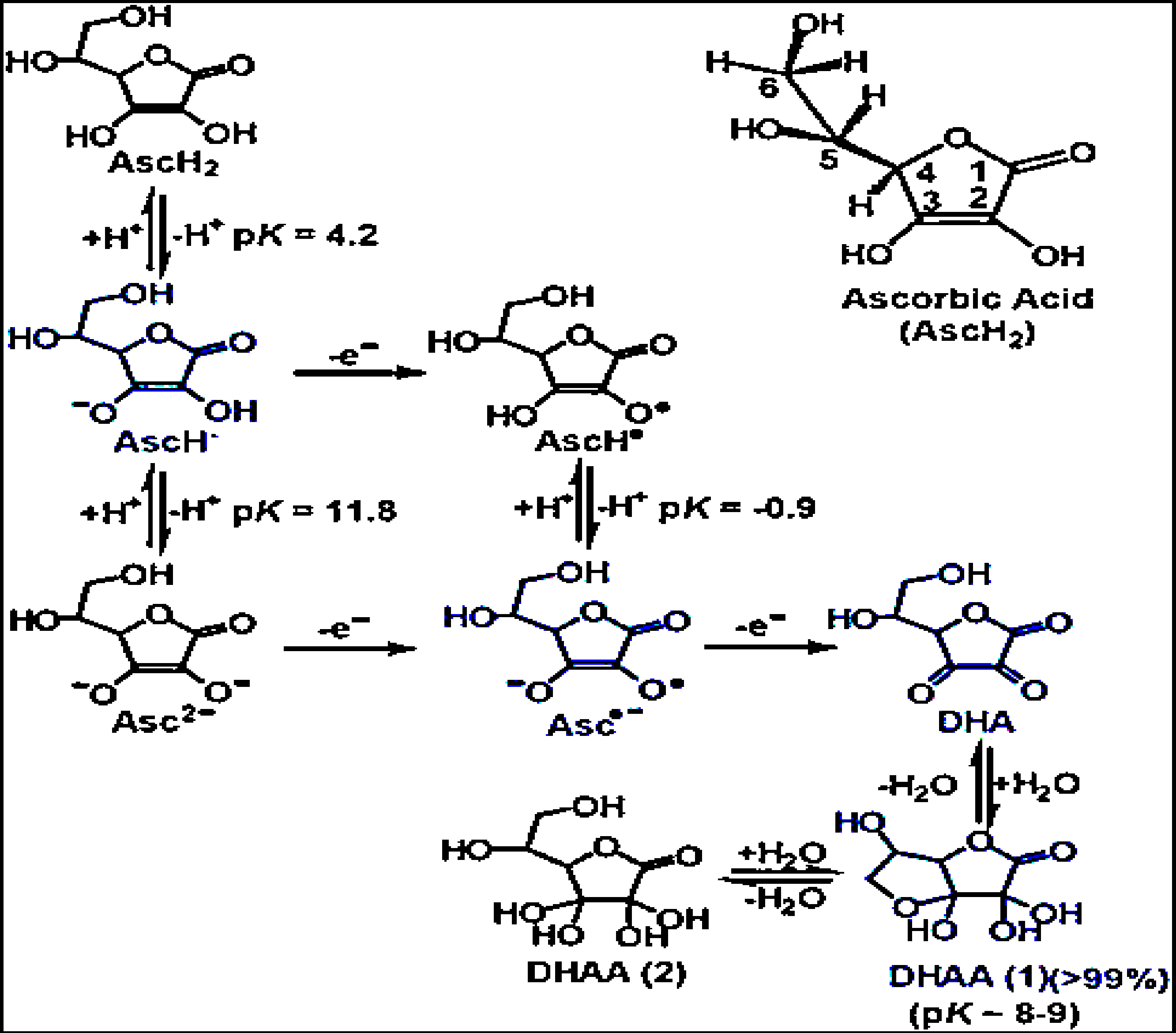
Figure 24: Proposed Structure for Ascorbate-Metal Complex $M(Asc)_2$



M = Mn (II), Fe (II), n =2
M=Ni(II), n = 0

Figure 25: Proposed Structure for Ascorbate-Metal Complex $M(Asc)_2(H_2O)_n$

The Basics of Ascorbic Acid Chemistry



<u>Source</u>:

Du, J., Cullen, J.J. and Buettner, G.R. (2012). Ascorbic acid: chemistry, biology and the treatment of cancer. *Biochimica et biophysica acta,* **1826,** 443-457.

Important Chemical Attributes of Ascorbate

- Both Ascorbic Acid (a di-acid) and Sodium Ascorbate (a mono-acid) possess *two reducing equivalents*, *in vivo*.

- Because the pKa values of Ascorbic acid are 4.2 and 11.8, Ascorbic acid ($AscH_2$) exists primarily as an Ascorbate anion ($AscH^-$), at physiological pH (SOURCE: Du *et al*. 2012).

- $AscH_2$ readily oxidizes to Dehydro-ascorbate (DHA) in a two step reaction:

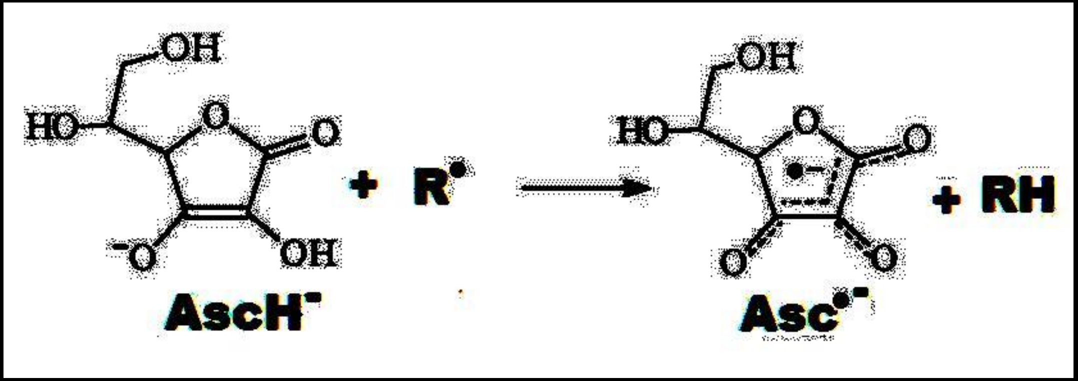
  (a) *Ascorbate anion ($AscH^-$) looses one electron*, forming the *Ascorbate radical*,
  (b) *Ascorbate radical* can loose another electron becoming *DHA*

- Ascorbate radical has several possible fates *in vitro*.

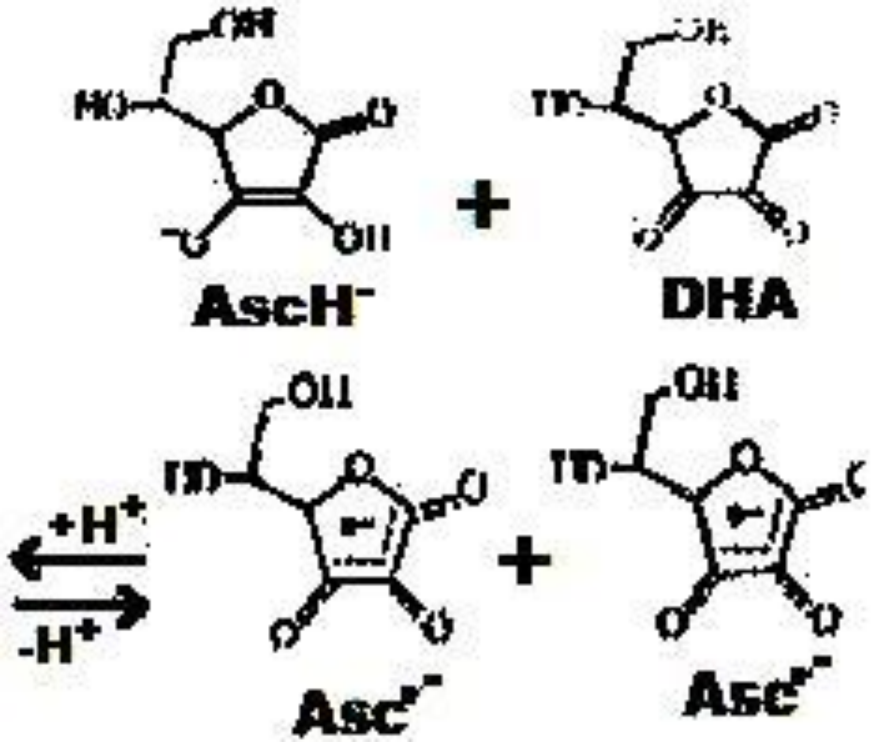
  (a) *It can loose another electron* and become DHA,
  (b) gain an electron and proton to regenerate the Ascorbate anion ($AscH^-$),
  (c) react with another radical and form a stable compound, or
  (d) two ascorbate radicals can dismutate to form one Acorbate anion and one DHA molecule.

SOURCE: http://www.sigmaaldrich.com/life-science/cell-culture/learning-center/media-expert/ascorbate.html#Chemical_Attributes

Two Major Routes to the Ascorbate Radical



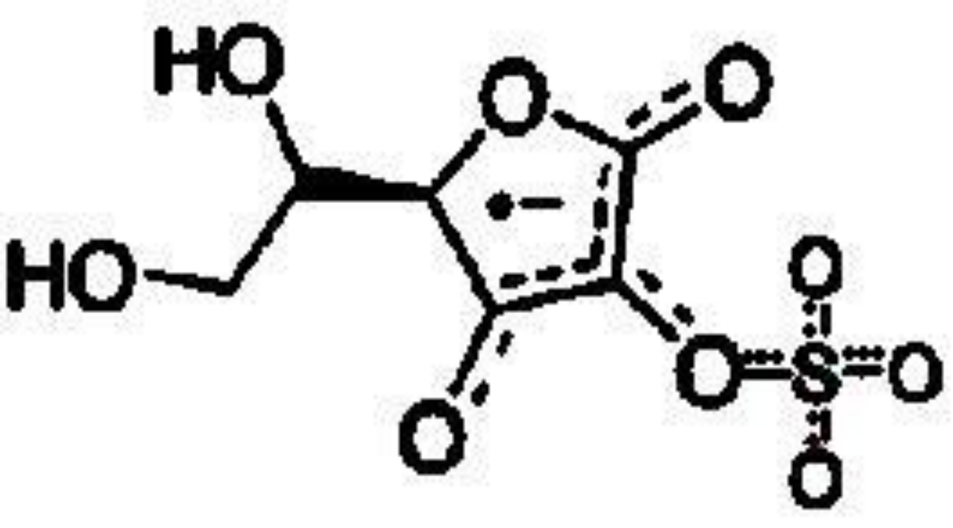
Radical-Scavenger Route



Dismutation Route

Our Proposal for a Universal Sulfurylation Factor

WE PROPOSE: 2-O-sulfate L-ascorbate radical plays a central role in the "universal nonspecific mesenchymal reaction", which has also been referred to as the Sanarelli-Shwartzman phenomenon.



- Chiral
- Paramagnetic
- Quasi-aromatic

- Takebayashi, J., Kaji, H., Ichiyama, K., Makino, K., Gohda, E., Yamamoto, I. and Tai, A. (2007). Inhibition of free radical-induced erythrocyte hemolysis by 2-O-substituted ascorbic acid derivatives. *Free Radical Biology and Medicine,* **43,** 1156-1164.
- Verlangieri, A.J. and Mumma, R.O. (1973). In vivo sulfation of cholesterol by ascorbic acid 2-sulfate. *Atherosclerosis,* **17,** 37-48.
- Cabral, J., Haake, P. and Kessler, K. (1998). Rearrangement of 3-Acyl Derivatives of L-Ascorbic Acid. *Journal of Carbohydrate Chemistry,* **17,** 1321-1329.

Routes to the 2-O-Substituted L-Ascorbate Derivative Radicals

- Putative Direct S_RN_2 Route:

  Asc radical + PAPS → Asc 2-S radical + PAP
  Asc radical + ATP → Asc 2-P radical + ADP

- Takebayashi’s Indirect “slow and continuous” Radical-scavenger Route:

  Asc 2-S + R· → Asc 2-S radical + RH
  Asc 2-P + R· → Asc 2-P radical + RH

A Novel Alternative Route ?

Asc radical + endogenous electrophilic
"sulfuryl" donor

Sunlight

Nitric oxide synthase
(NOS)

Asc 2-S radical

Acidolysis, defined:

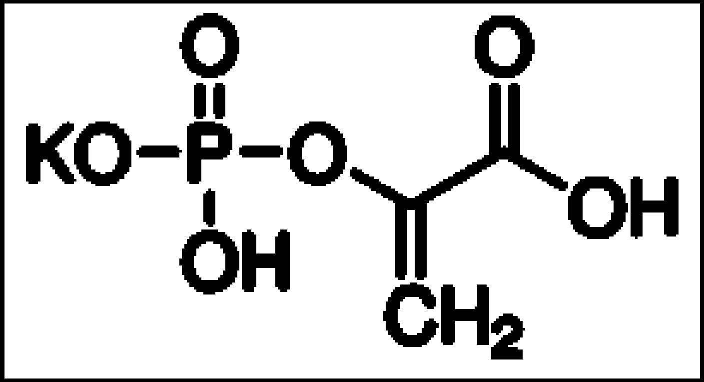
<u>ACIDOLYSIS</u>: noun ac·i·dol·y·sis \ˌa-sə-ˈdä-lə-səs\
“any chemical reaction analogous to hydrolysis in which an acid plays a role similar to that of water”

Some Important Corollaries to our Hypothesis:

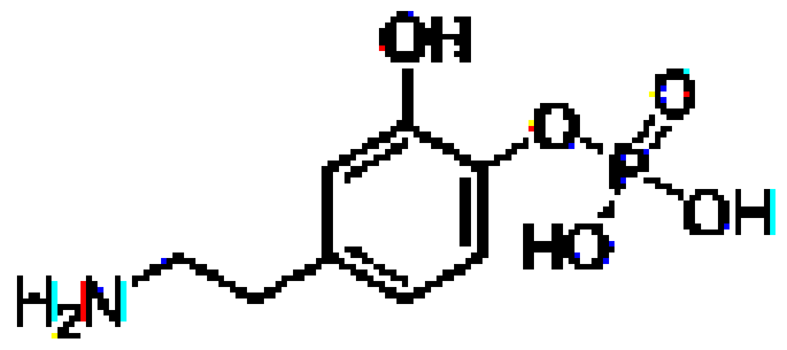
- ‘Hydro’-lases can act as ‘transfer’-ases in a relatively hydrophobic environment. Acidolysis is catalyzed by the Ascorbate radical during inflammatory stress!

- The <u>hydrophobic effect</u> and <u>heterogeneous catalysis</u> enable the 2-O-substituted-L-ascorbate radicals to “acidolyze” biological nucleophiles. Consider <u>lipases</u>, <u>phosphatases</u>, <u>sulfatase</u>s, behaving in an anabolic capacity during the SSP.

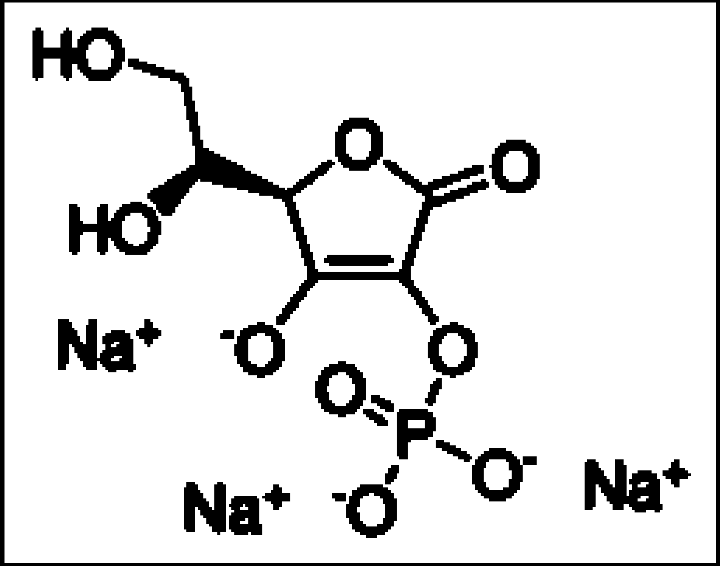
What do they have in common?



Phosphoenol pyruvate

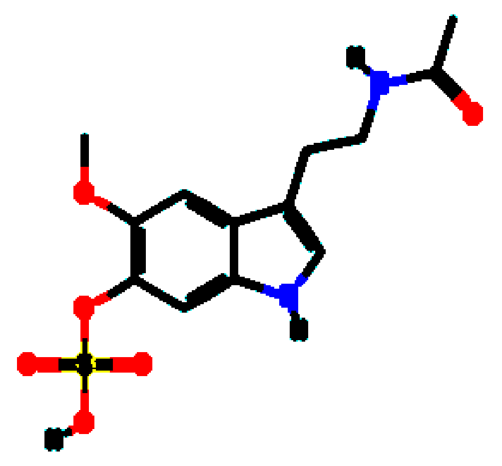


Dopamine-4-phosphate ester

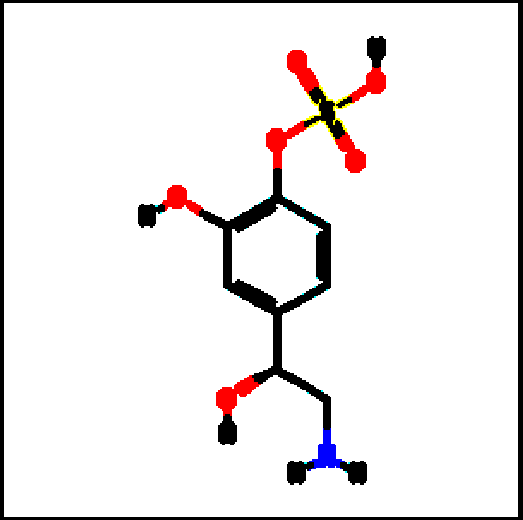


2-O-Phosphate-L-ascorbate

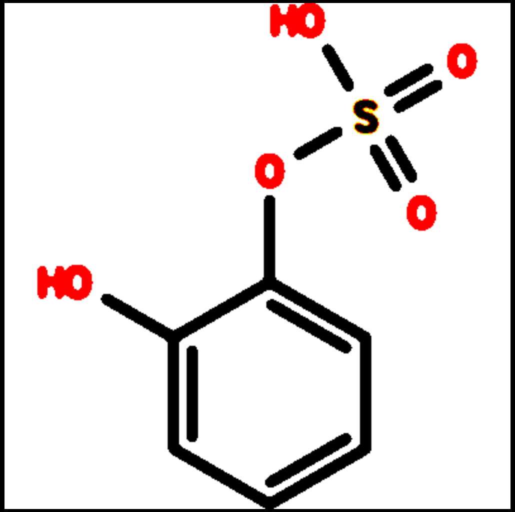
What do they have in common?



Noradrenaline sulfate

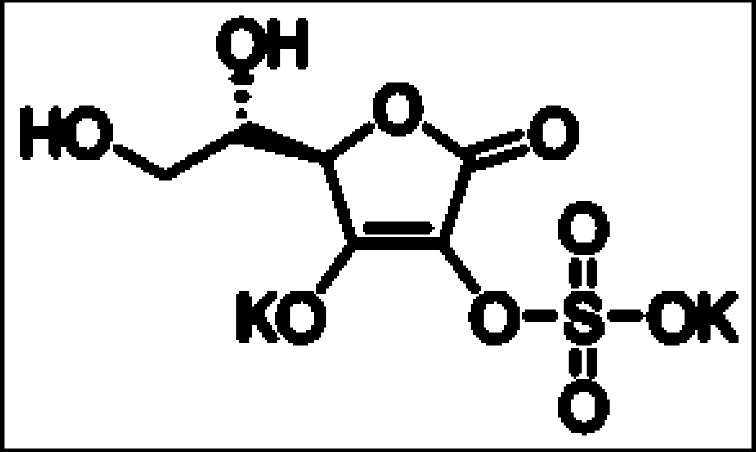


Melatonin sulfate

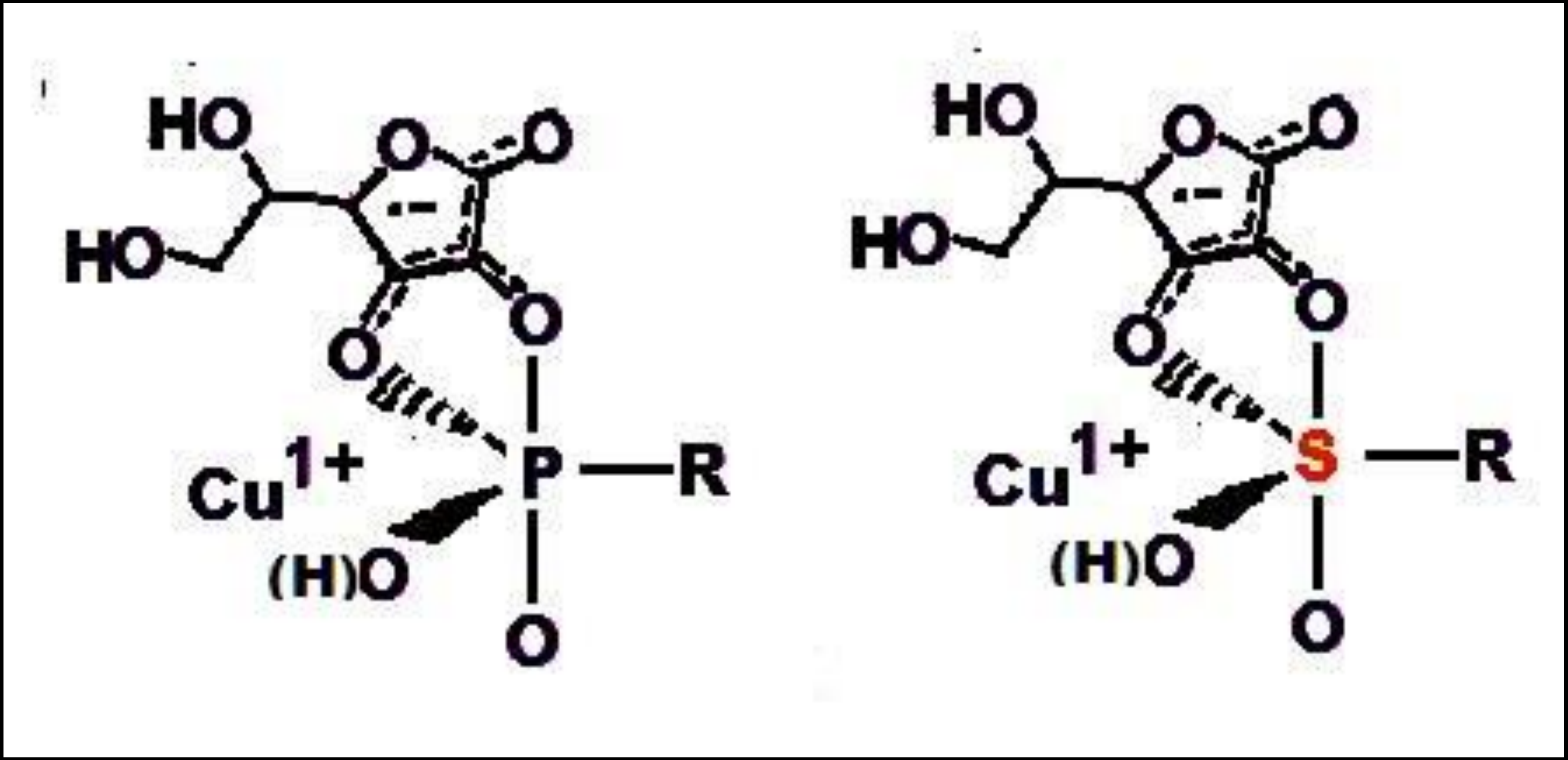


Catechol sulfate

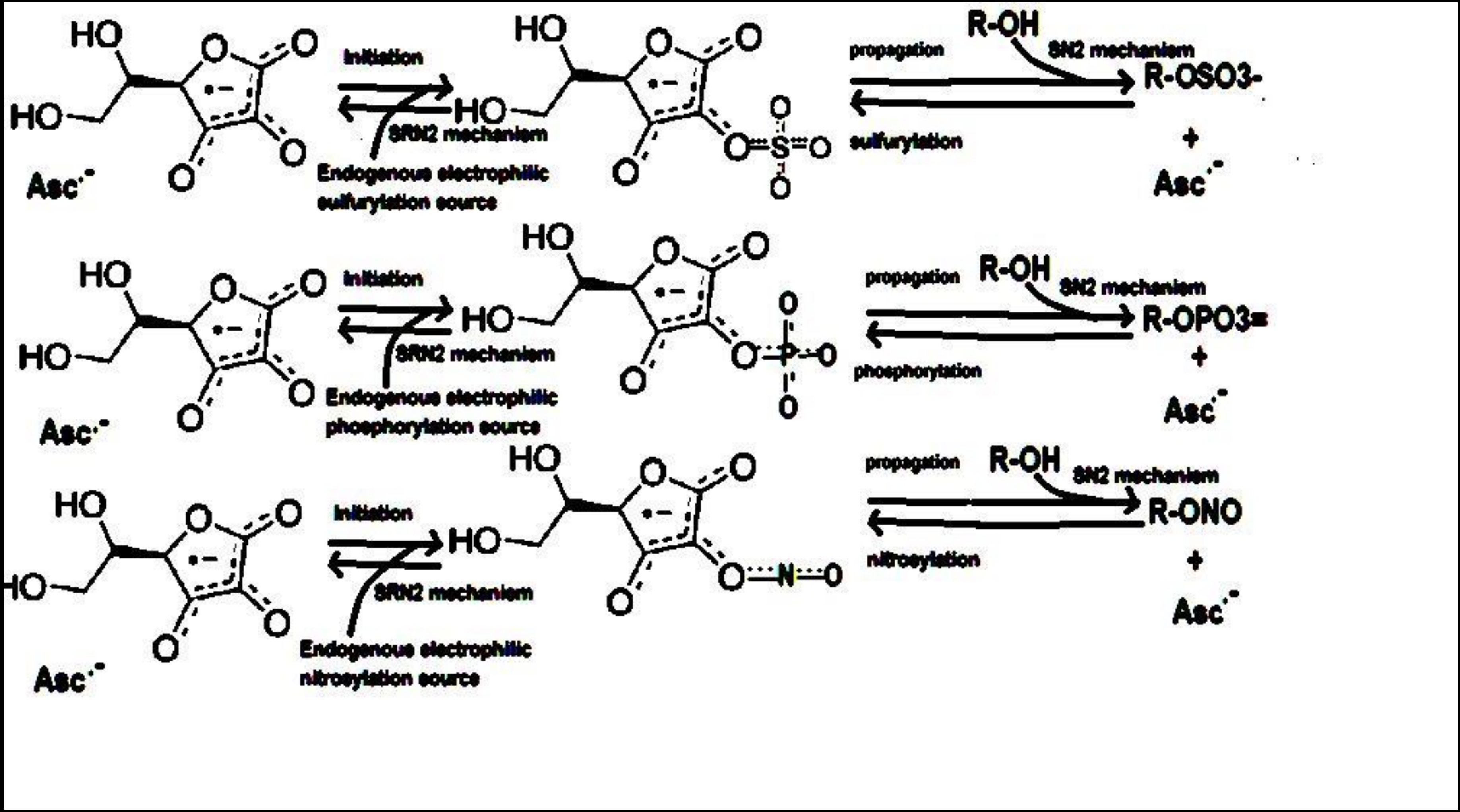
2-O-Sulfate L-ascorbate



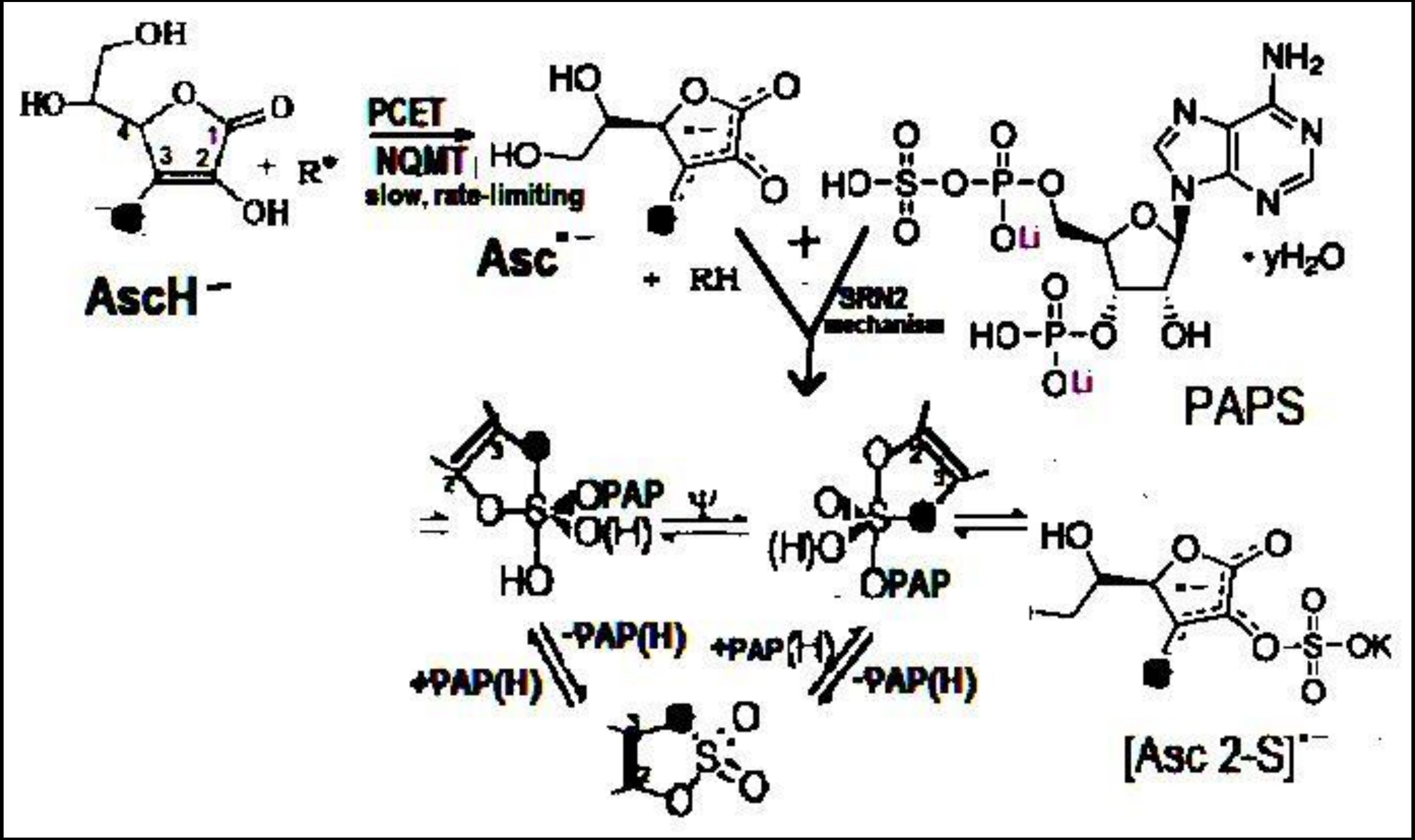
Putative <u>Ascorbate Derivate radicals</u> as "reactive transient" group transfer factors



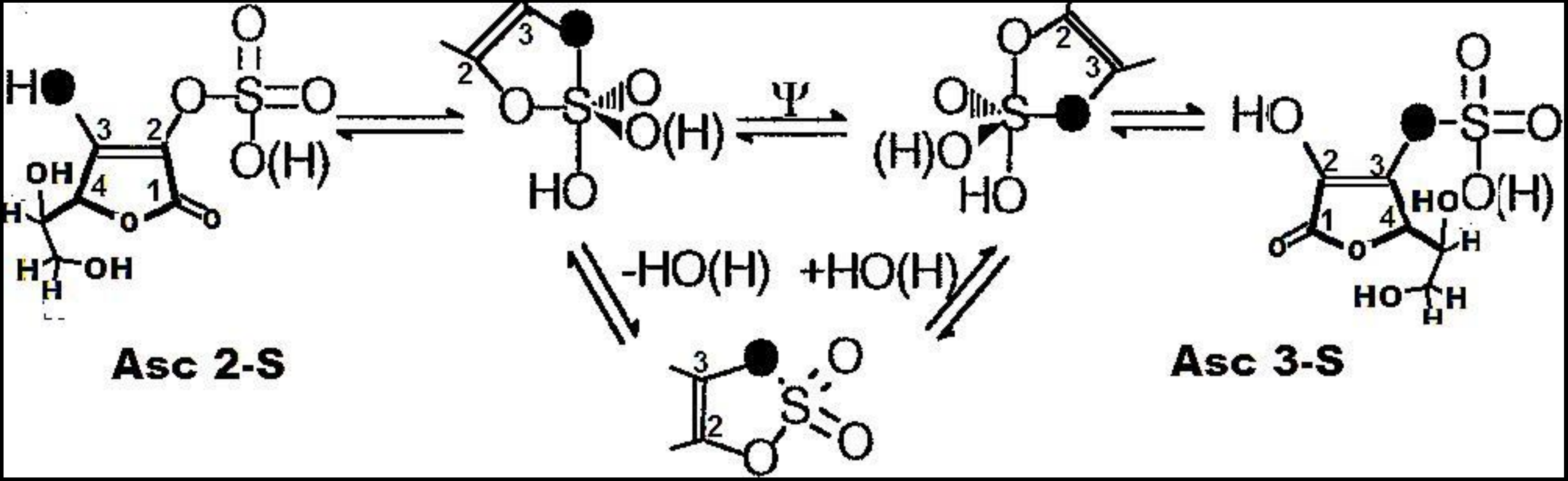
Initiation, Propagation, Termination of Radical Chain Reactions



Preformed ATP and PAPS as "penultimate" electrophilic phosphoryl and sulfuryl sources

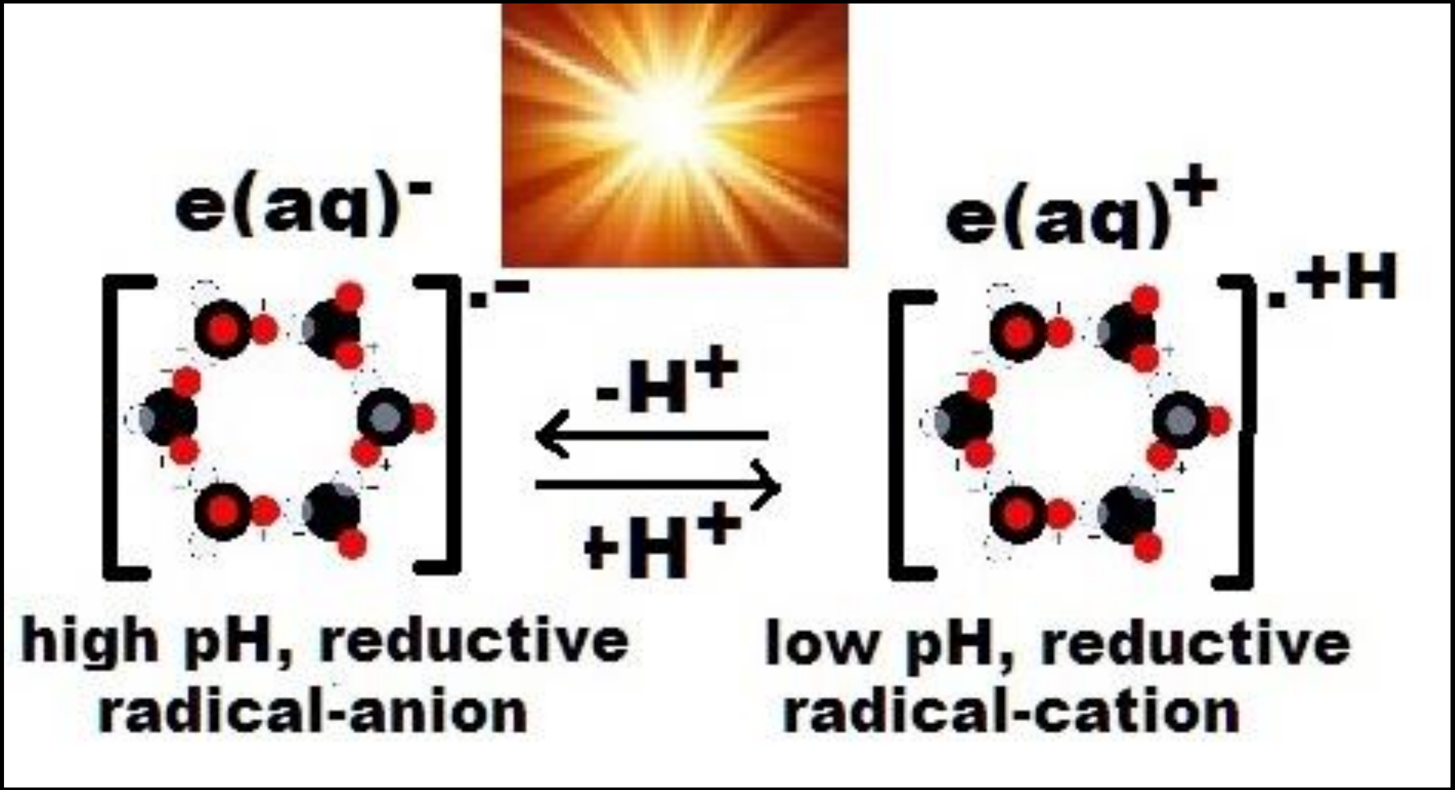


How to "flatten the free energy landscape" in biological group transfer reactions



Berry pseudorotation; spin-correlated radical pairs; pH-dependent speciation of e aq's; e aq's "assist“; ELF EM energy "assist“; Cu^{1+} "assist"

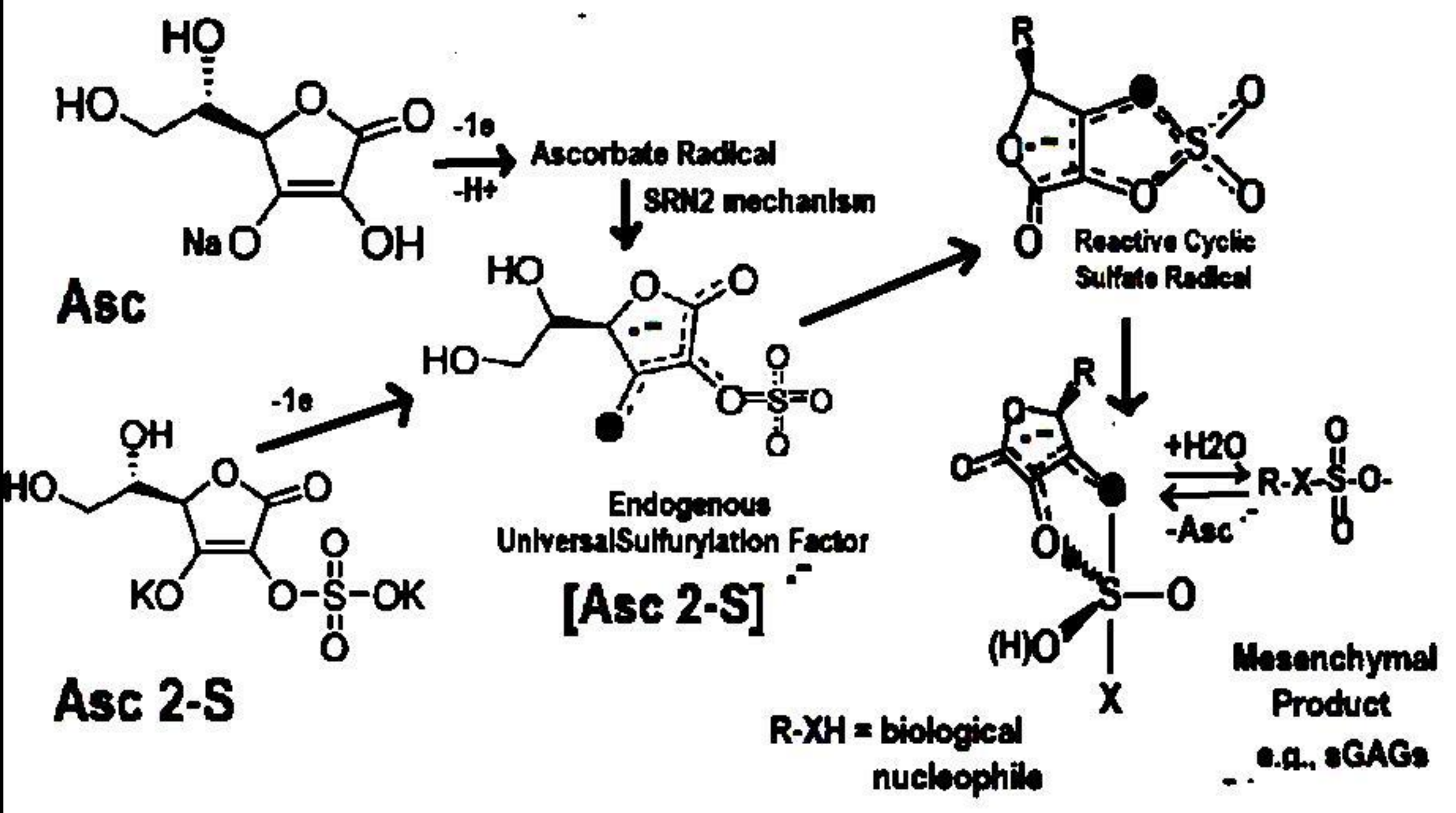
Our Proposal for pH Dependent e(aq) Speciation



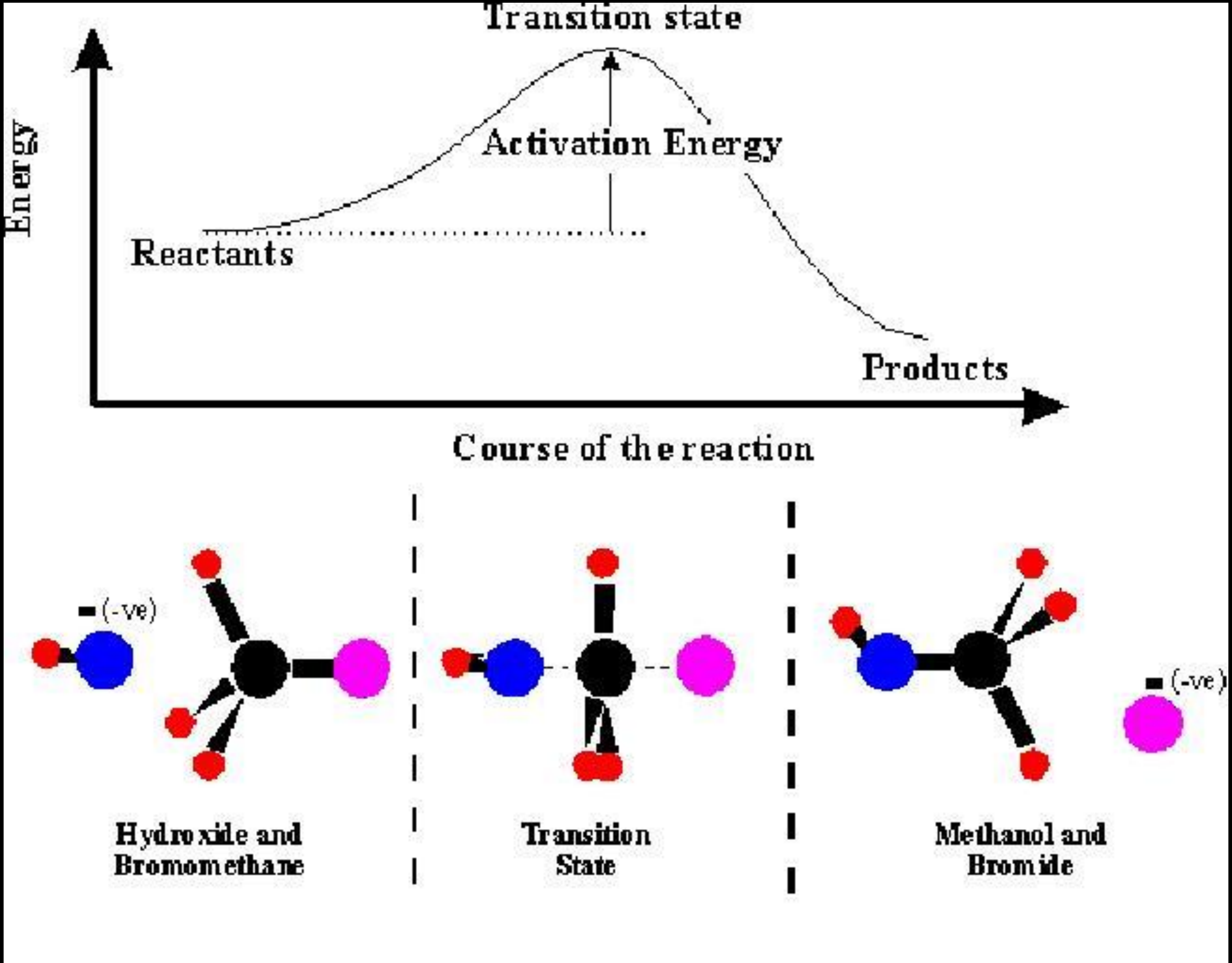
Mizuse, K. and Fujii, A. (2013). Characterization of a Solvent-Separated Ion-Radical Pair in Cationized Water Networks: Infrared Photodissociation and Ar-Attachment Experiments for Water Cluster Radical Cations (H2O)n+ (n = 3–8). *The Journal of Physical Chemistry A,* ***117, 929-938.***

Larsen, R.E., Glover, W.J. and Schwartz, B.J. (2010). Does the hydrated electron occupy a cavity? *Science,* ***329, 65-69.***

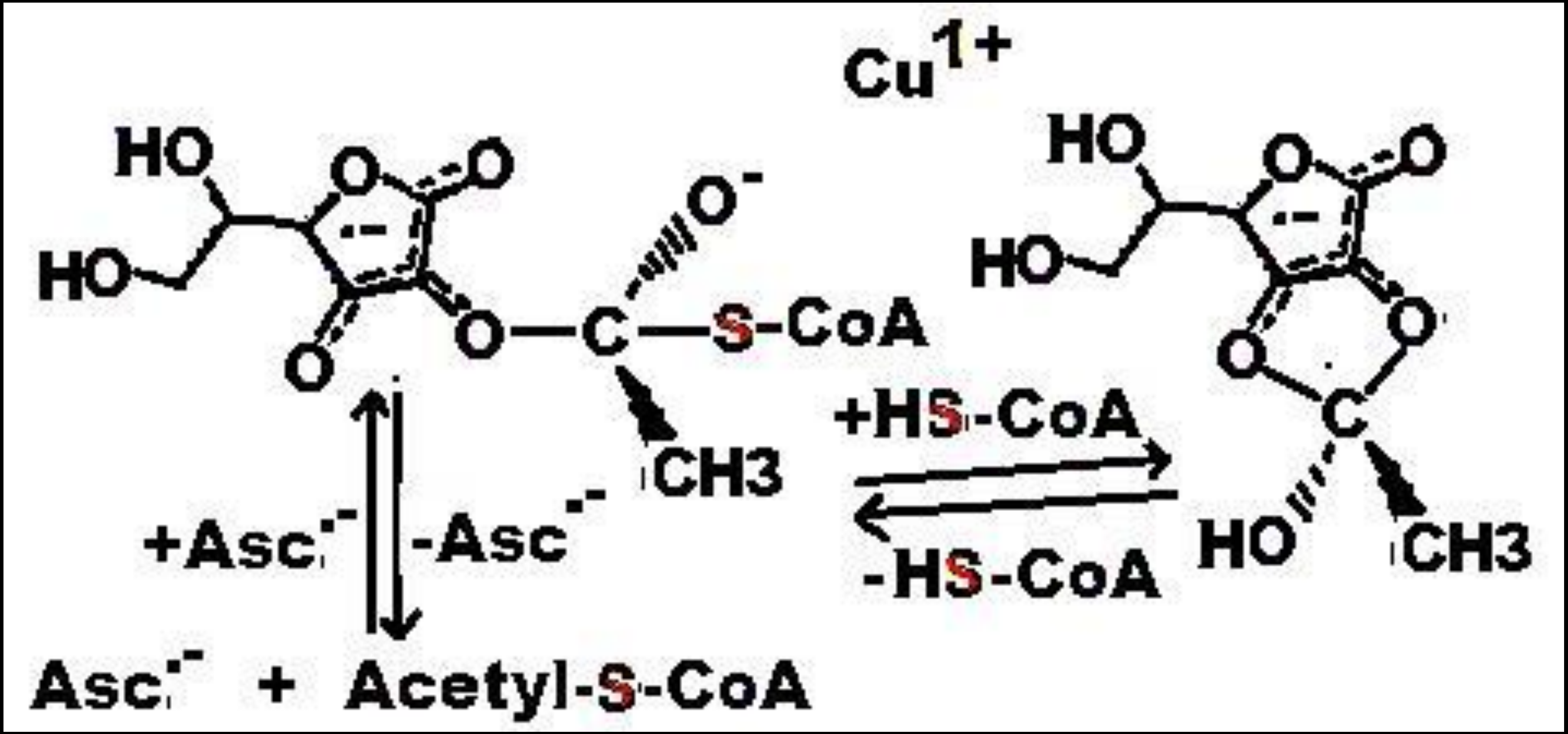
Proposed Route to Acid Mucopolysaccharides in the SSP



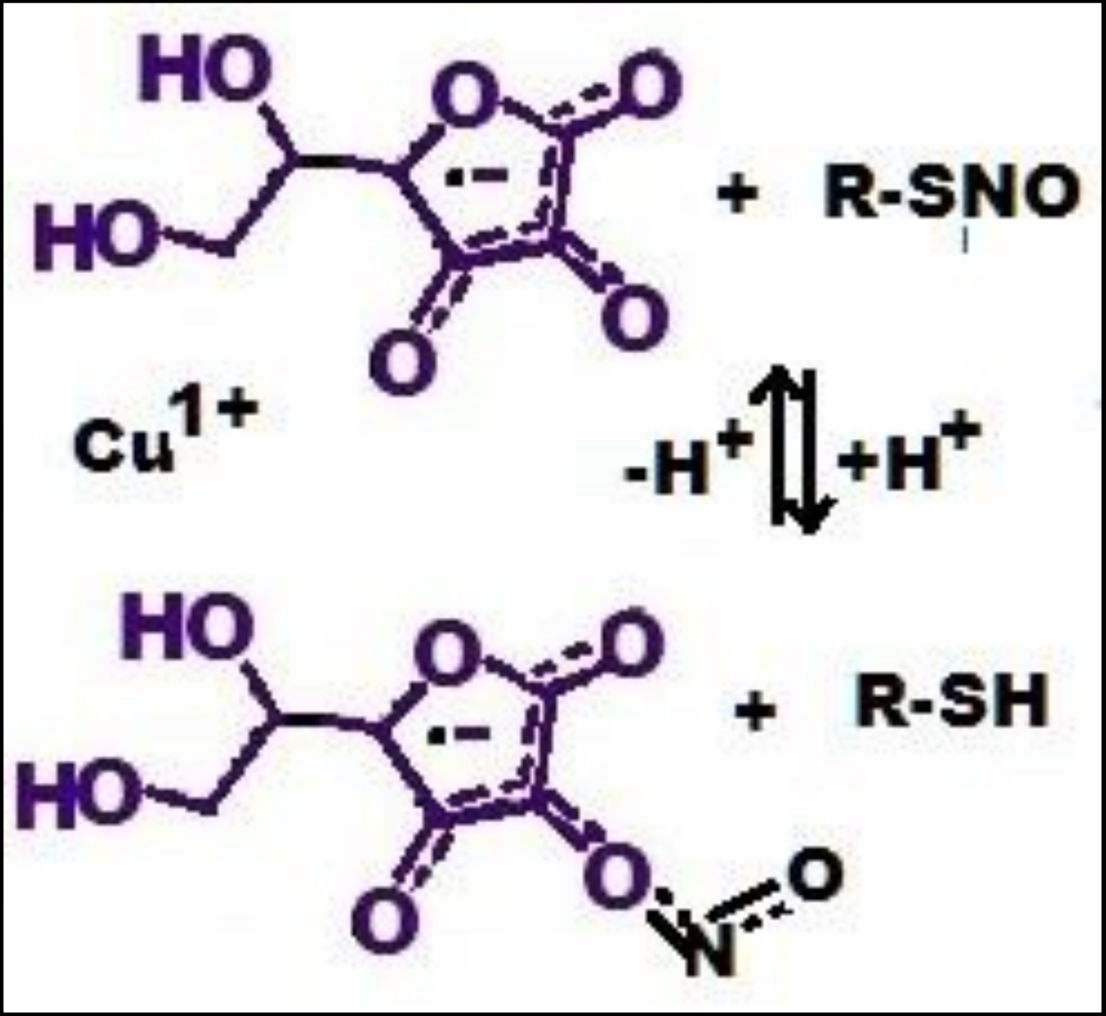
Transition-state Theory



Ascorbate Radical as a Transacylation Catalyst



Ascorbate Radical as a Transnitrosylation Catalyst



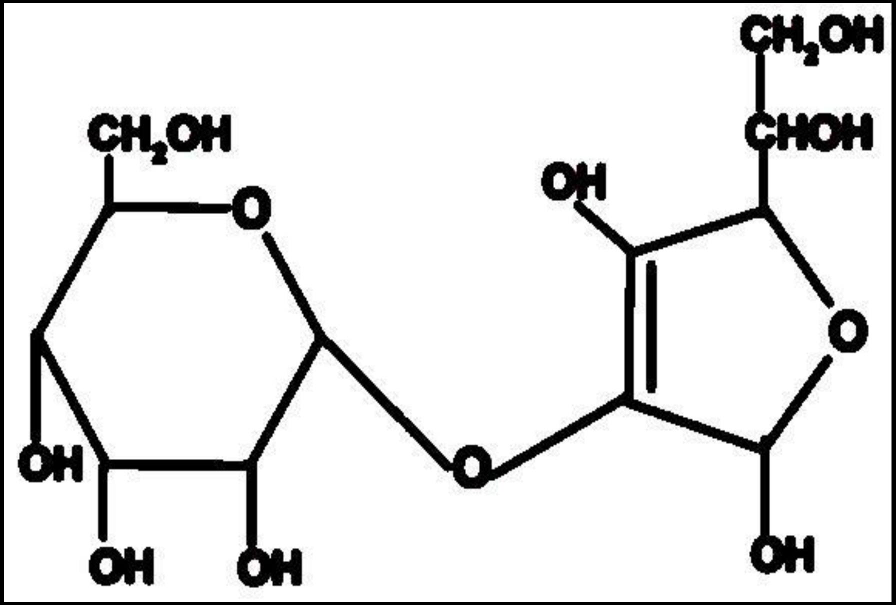
Ascorbate Radical as a Transglycosylation Catalyst

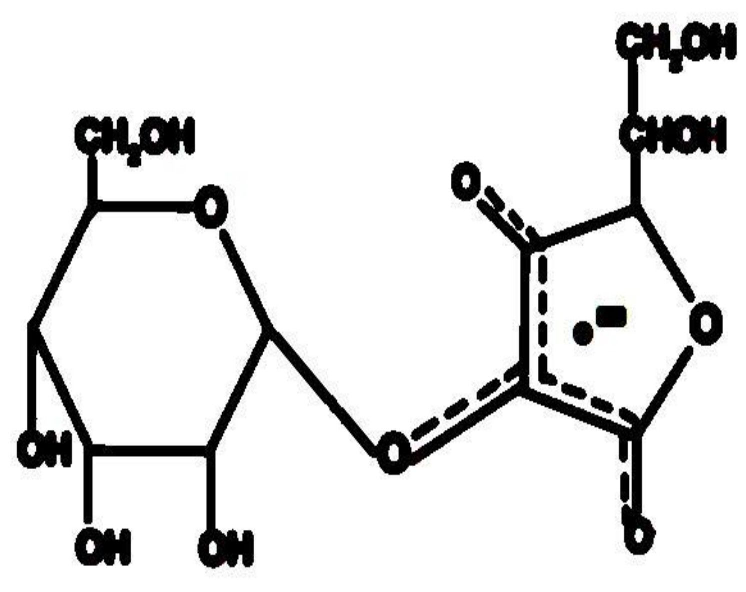
2-O-glucosyl- L-Ascorbate

(a natural product)

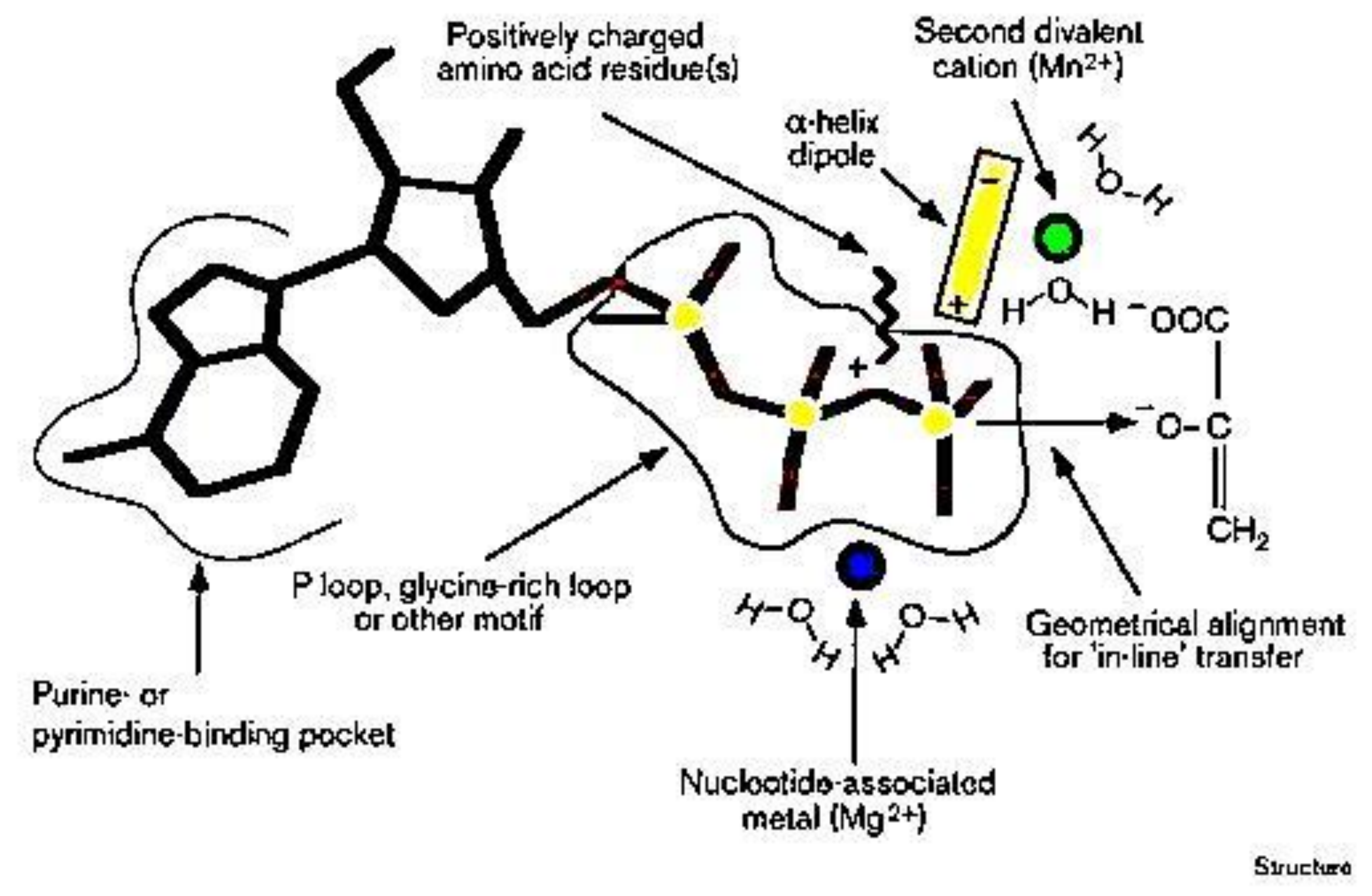
Proposed 2-O-glucosyl L-Ascorbate Radical

(group transfer factor)

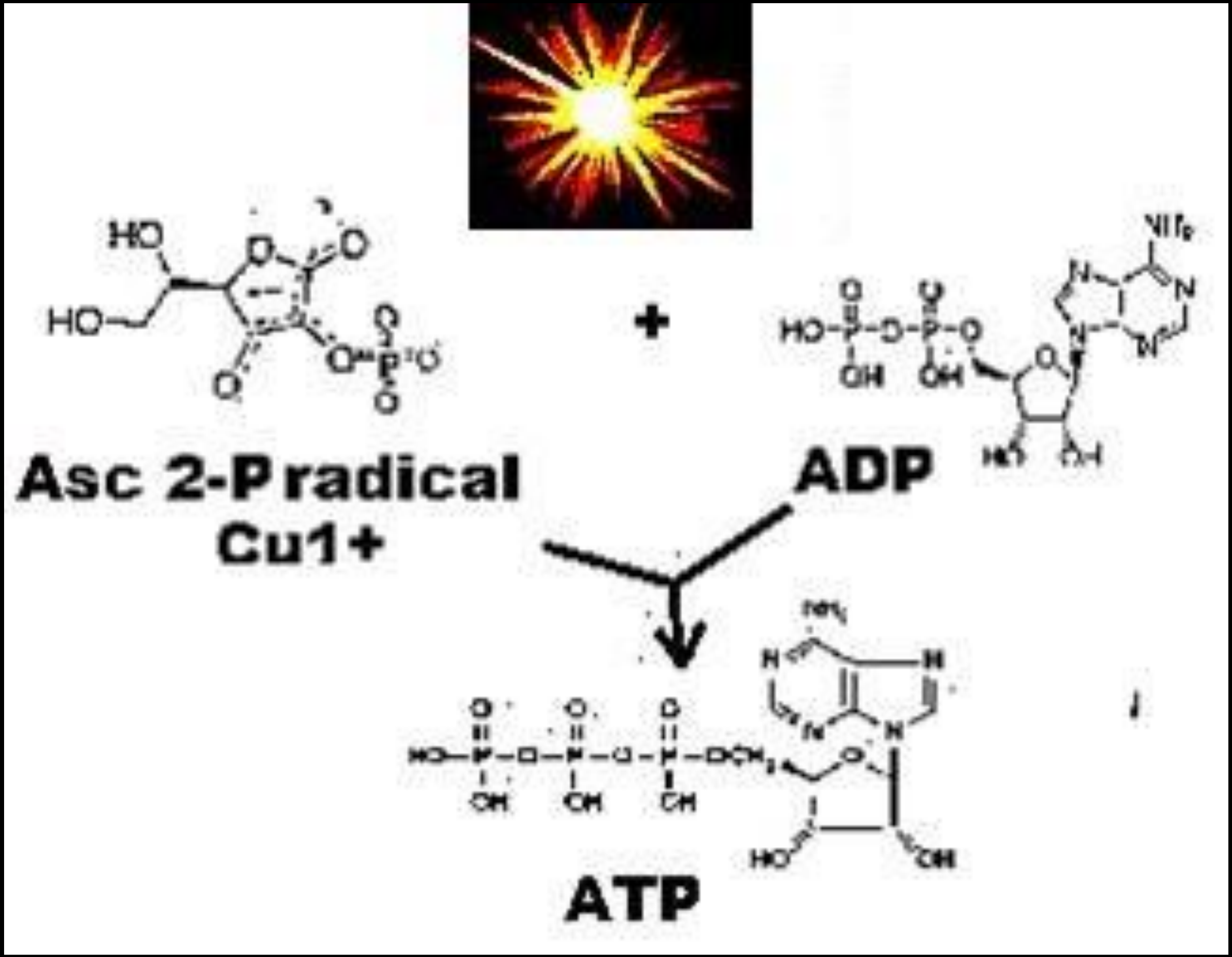




Pyruvate Kinase is Instructive



The Ascorbate Radical as a Photophosphorylation Catalyst ?



Conclusion:

The Ascorbate radical, and the putative Ascorbate derivative radicals, provide:

(a) lifelong biological group transfer catalysis, in inflamed tissue,

(b) and lifelong modulation of redox kinetics

THANK YOU !