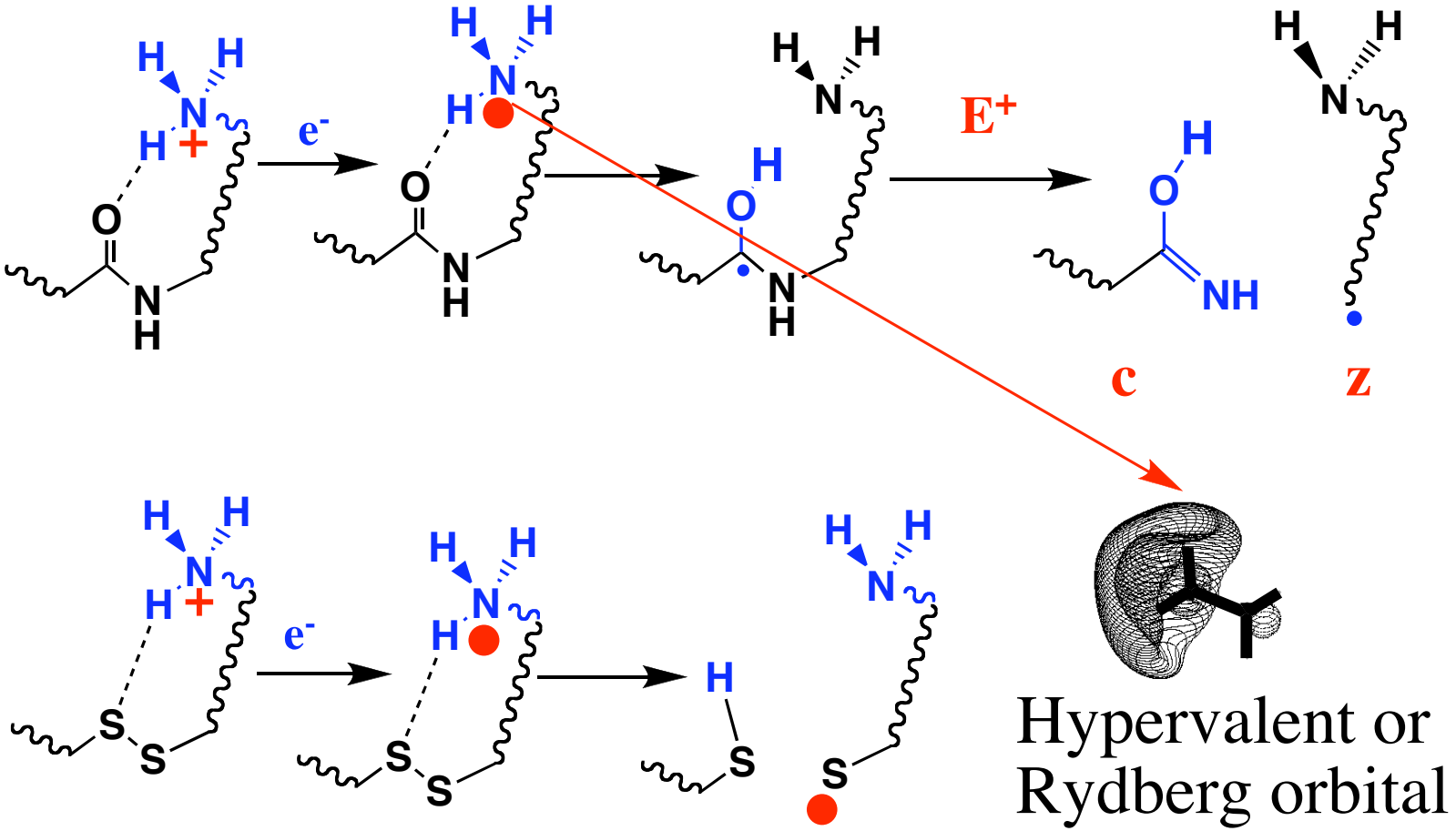
Mechanisms for peptide S-S and N-C_α bond cleavage in ECD/ETD mass spectroscopy- Anions in Disguise

Jack Simons
University of Utah

Funding: NSF Analytical and Surface Chemistry
My main collaborators: P. Skurski, M. Sobczyk, D.Neff
http://simons.hec.utah.edu for references

How does ECD (or ETD) fragment peptides?

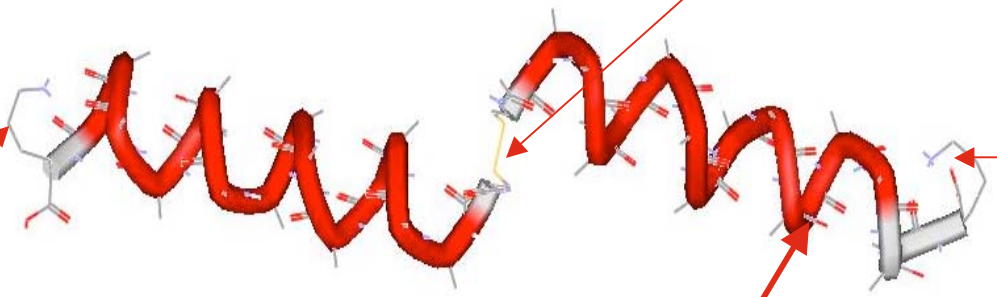
McLafferty and Zubarev (Cornell) suggested the following:



This requires the + site to be close to the carbonly group or S-S so the H atom can "find" the C=O π bond or S-S σ bond.

What is the evidence against the Cornell mechanism?

Marshall Group ECD of doubly charged disulfide-linked dimers $(AcCA_NK+H)_2^{2+}$ and $(AcCA_NK+Na)_2^{2+}$ produced S-S bond cleavage when N = 10, 15, or 20 (distance between SS bond and + sites = 15, 24, 33 Å).



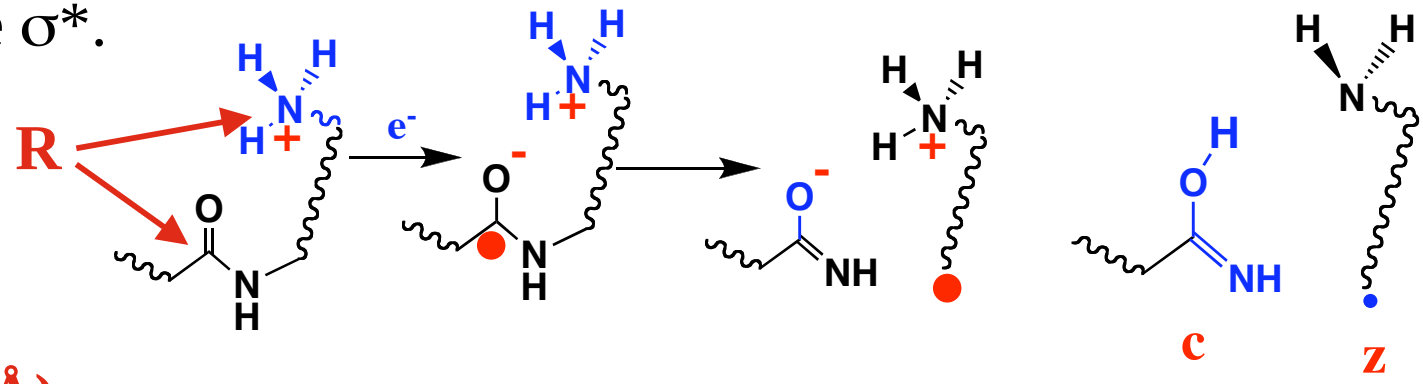
Positively charged lysine

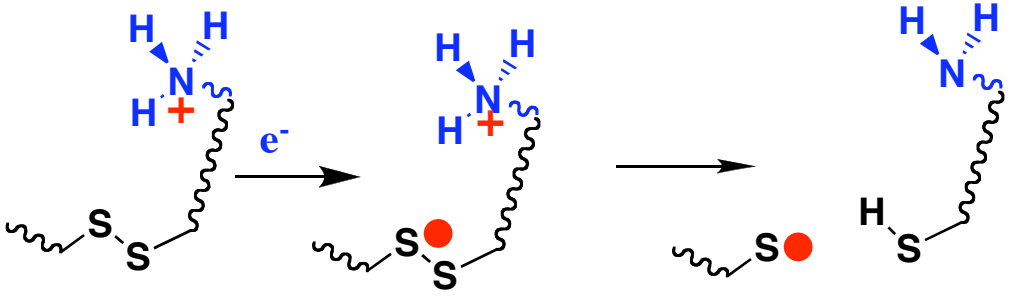
Positively charged lysine

1. How does the H atom "find" the S-S bond so far away?
2. Na atoms don't attack and cleave S-S bonds, so how does $(AcCA_NK+Na)_2^{2+}$ cleave?
3. Why does only limited (the 4 amino acids closest to each Lys) N-C_α cleavage occur in $(AcCA_NK+H)_2^{2+}$?

We suggested that an electron could attach (or transfer) exothermically to an OCN π* (or SS σ*) orbital if there were enough Coulomb stabilization at the π* or σ* site. Ca. 2.5 eV for the π*, ca. 1 eV for the σ*.

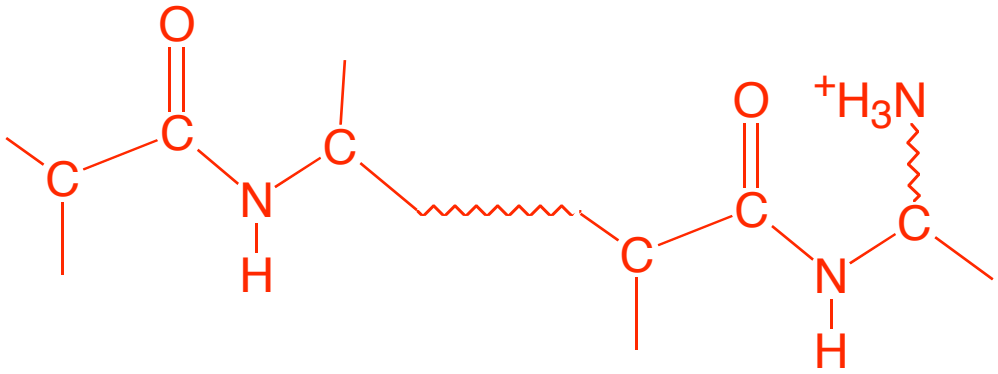
Coulomb stabilization = 14.4eV/R(Å)





Our mechanism does not require the + site to be as close (e.g., within H-bonding) as in the Cornell mechanism.

How does this Coulomb stabilization work?



OCN π*-site potential in absence of positive charges gives metastable π* state

2.5 eV

Potential energy

0.0

4 eV

-NH_3^+ site potential also can bind the electron

OCN π*-site potential in the presence of positive charges giving bound π* state.

So, our Coulomb model predicts that S-S cleavage will occur as long as R < 2x14.4eVÅ/1eV= 29 Å. Pretty much as observed.
It also says that N-C_α cleavage can occur within 14.4eVÅ/2.5eV = 6 Å of the termini. Also, much as observed.

Coulomb stabilization = 14.4eV/R(Å)

Key questions:

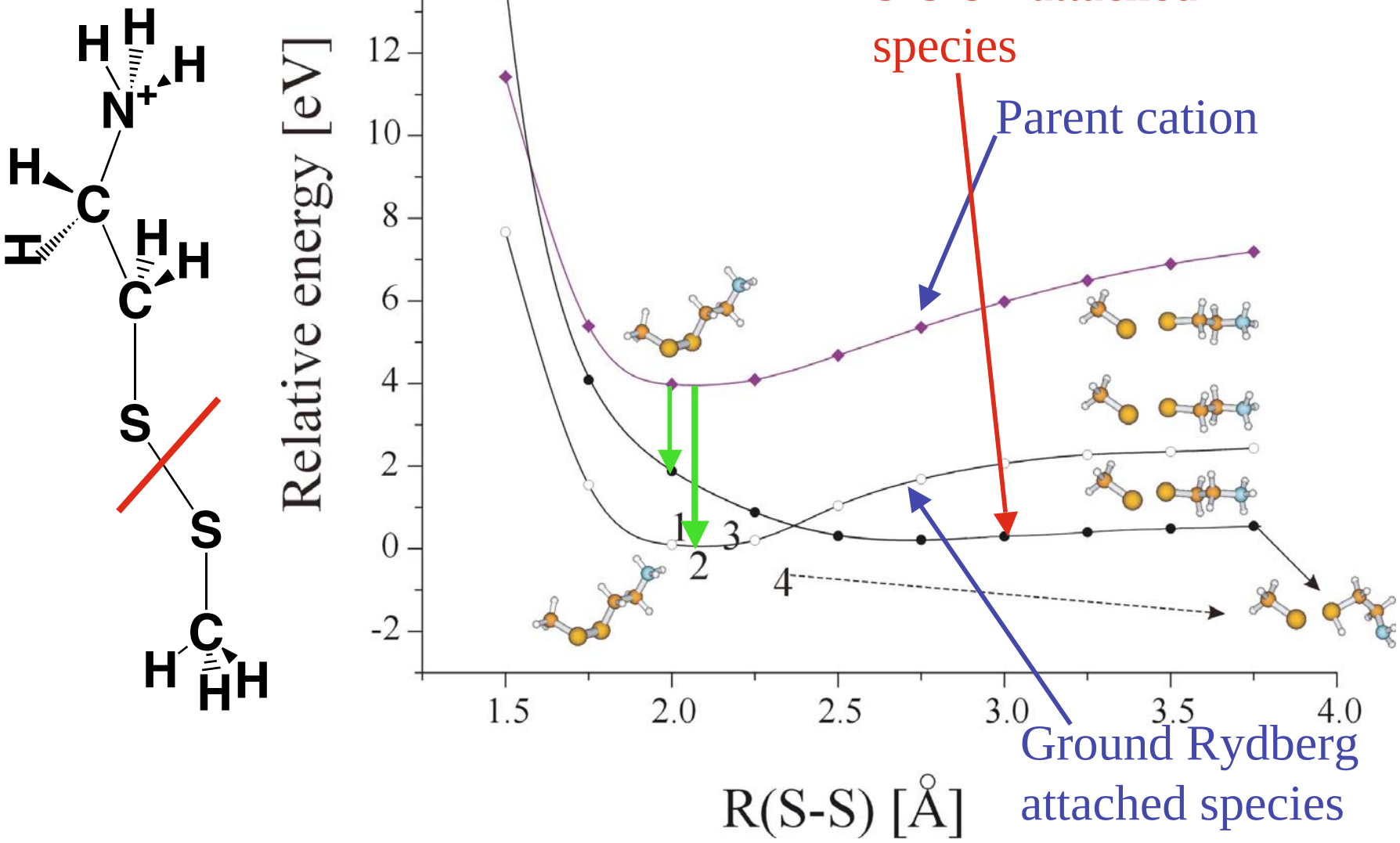
1. Does it make sense that an electron can exothermically attach to to an OCN π^* or S-S σ^* orbital?

2. Does the electron not **"prefer"** to attach to the positive $-NH_3^+$ site?

3. Can the electron attach to the + site and then **transfer** to the OCN or SS bond site?

Answers: Yes, Yes, Yes but it depends on the distances.

Where can an e^- attach and then what happens? S-S bond attachment case.



Does an electron not "prefer" to bind to the + site?

$\sigma = P \pi R^2_C$

$P = 2\pi H_{1,2}^2 (\hbar \Delta F\ v)^{-1}$

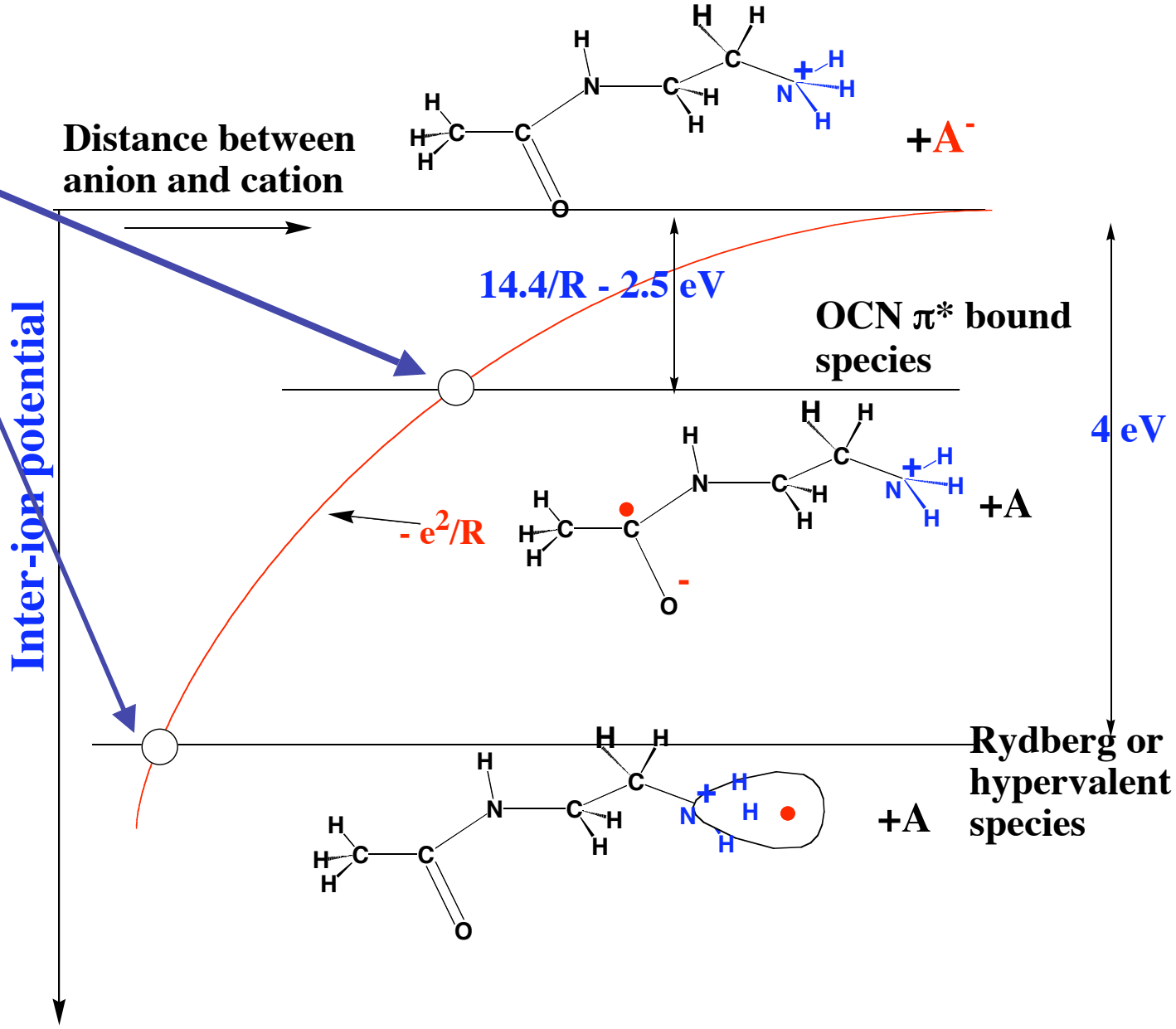
= 2.7 x10⁰ Å²

for Rydberg

= 3.2 x10⁻² Å²

for π*

So, yes, it prefers the + site.



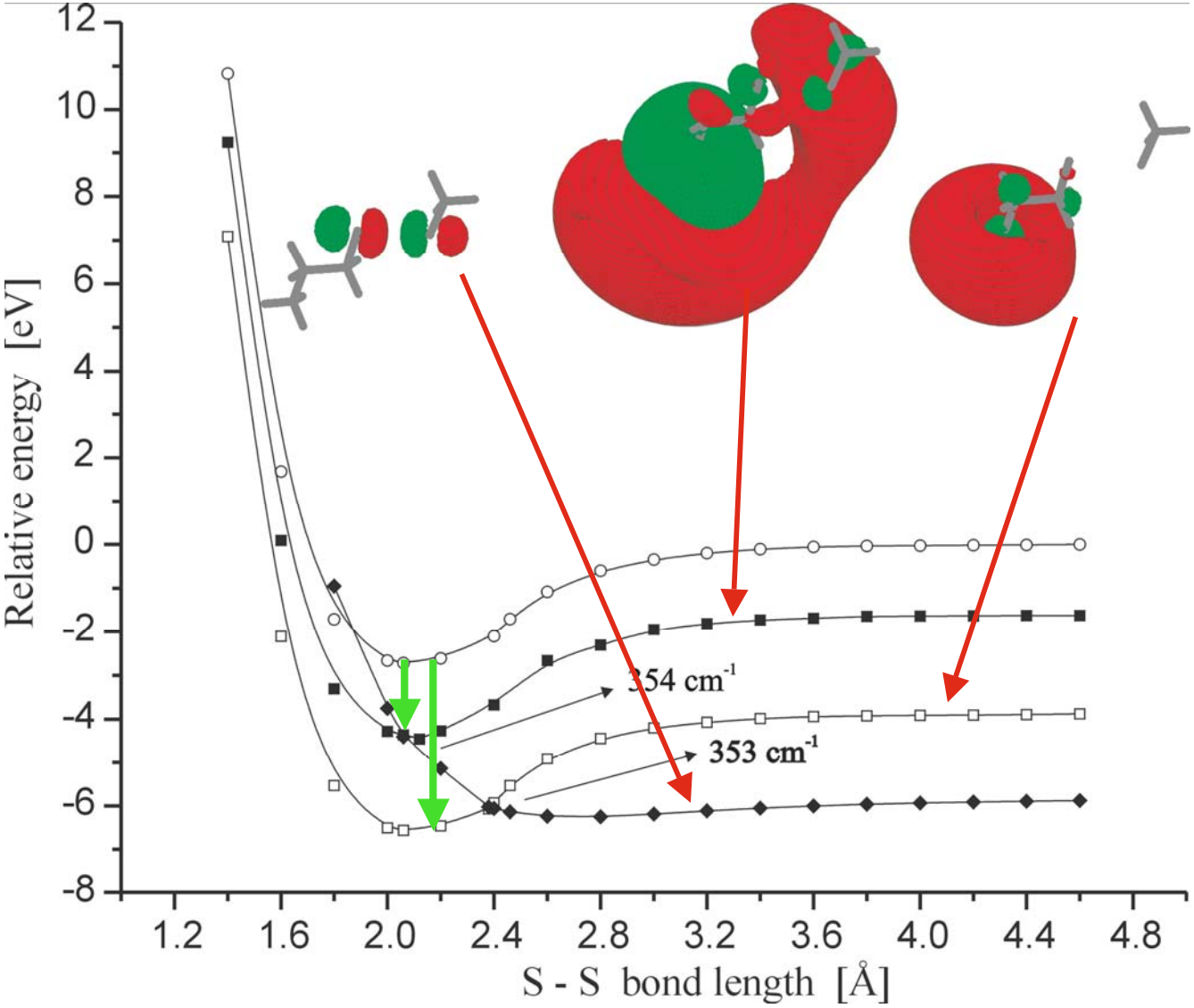
Key questions:

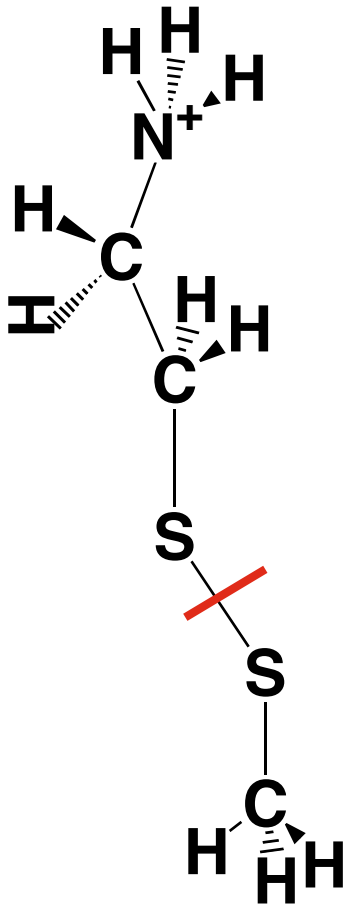
1. Does it make sense that an electron can exothermically attach to to an OCN π* or S-S σ* orbital? Yes, if there are positive charges in proximity. √

2. Does the electron not **"prefer"** to attach to the positive $-NH_3^+$ site? Yes, by a couple of orders of magnitude. √

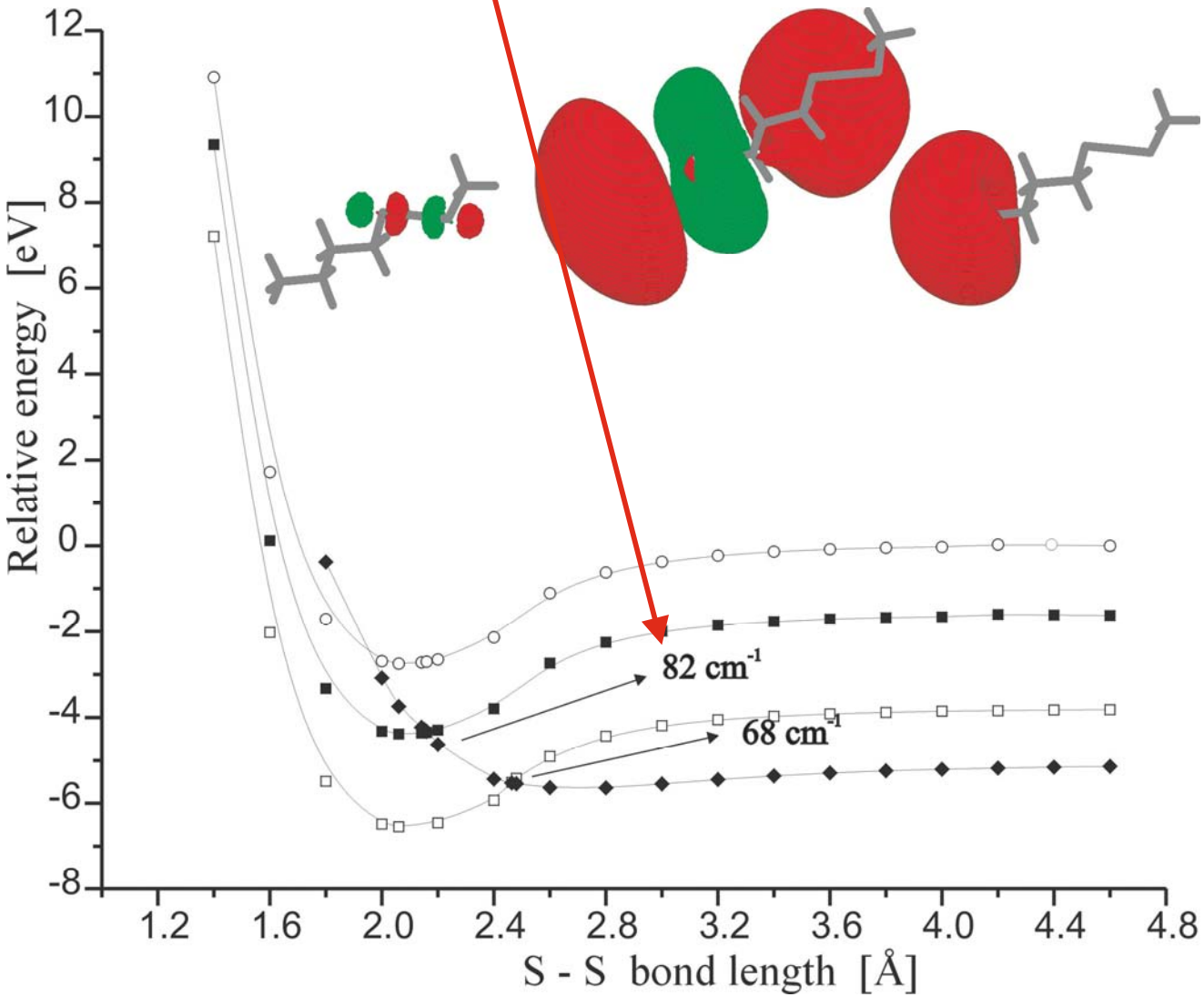
3. Can the electron attach to the + site and then **transfer** to the OCN or SS bond site?

Can an electron attach at the + site and then "transfer" through-bonds to a π* or σ* site?

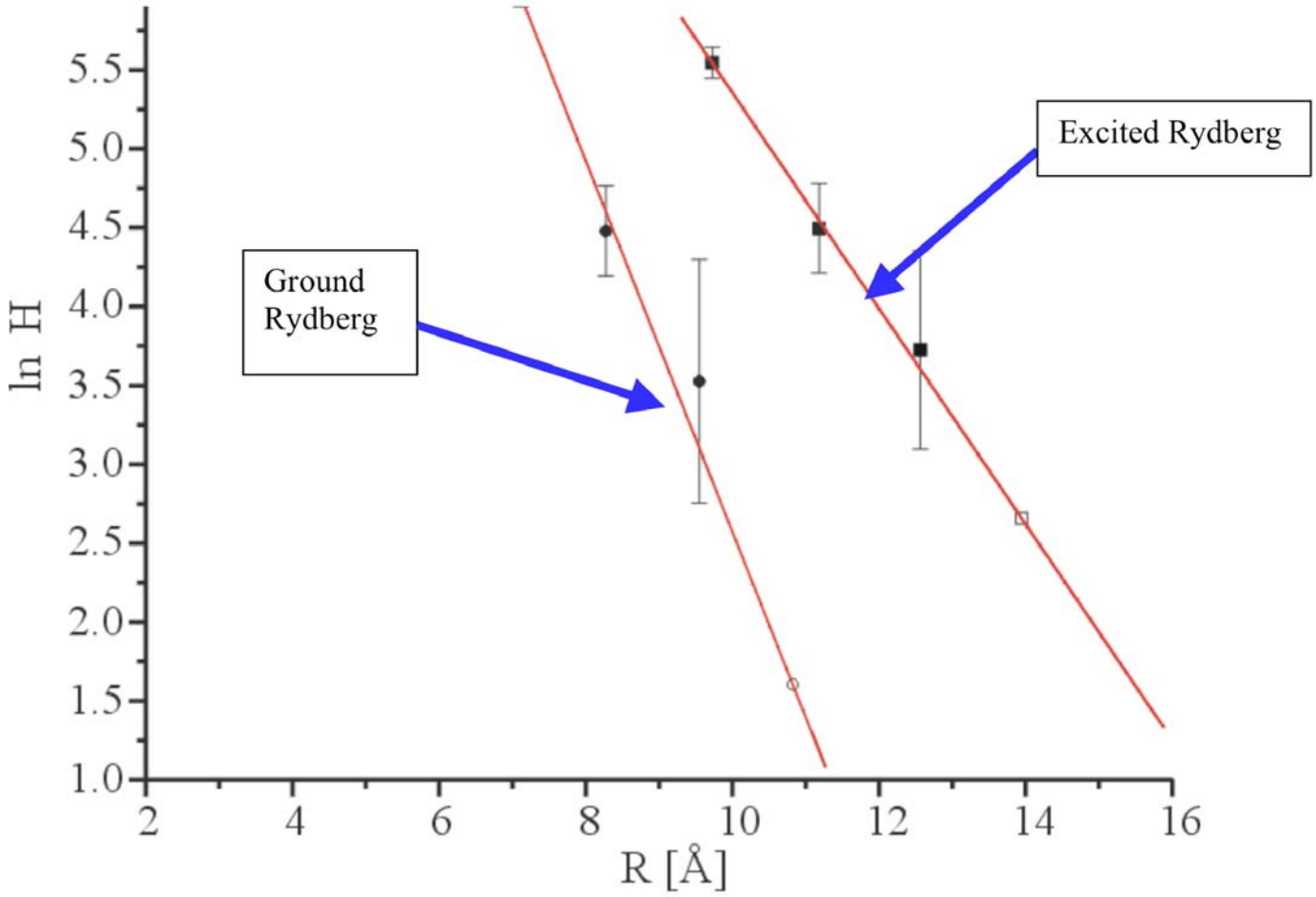




The $H_{1,2}$ couplings are small (10-1000 cm^{-1}) and decrease so rapidly with the distance R between the + and π^* or σ^* sites that transfer occurs only over 4-5 bonds.



As expected, the $H_{1,2}$ couplings fall off exponentially with distance.



This rapid decay is what limits through-bond transfer to 4-5 bonds.

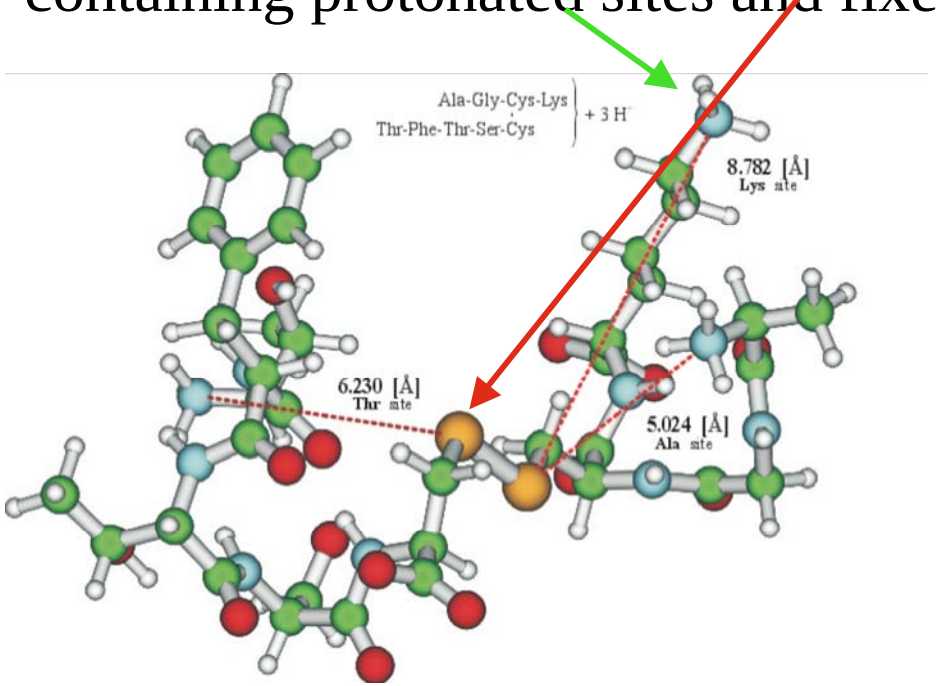
Conclusions:

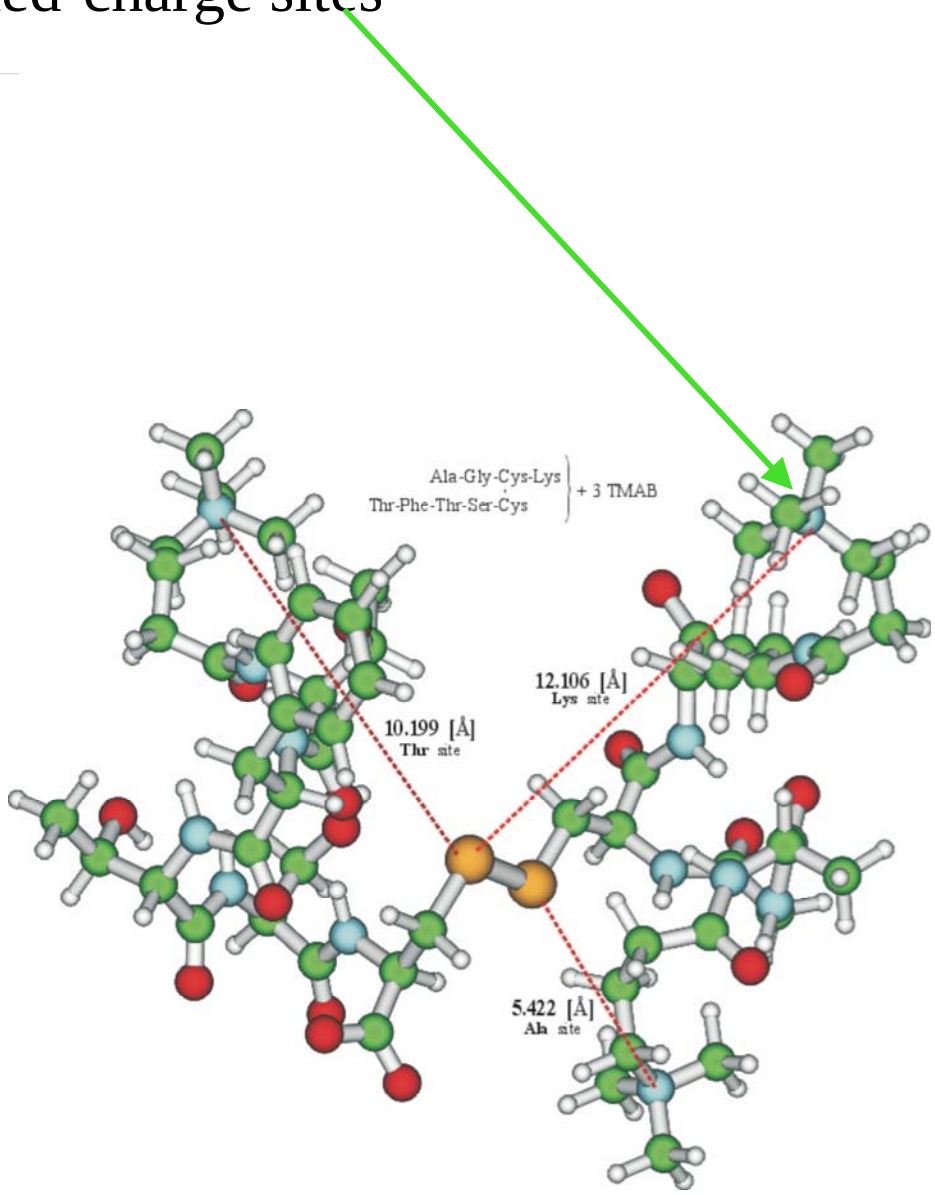
Electrons can attach either to + sites or, via Coulomb stabilization, to SS σ^* or OCN π^* sites.

In the latter cases, subsequent cleavage of the SS σ bond is prompt; cleavage of the N-C_α bond has a barrier of ca. 30 kcal mol^{-1}.

Electrons are more likely to attach at a + site, but then they can transfer only over ca. 4-5 bonds to reach an S-S or amide site.

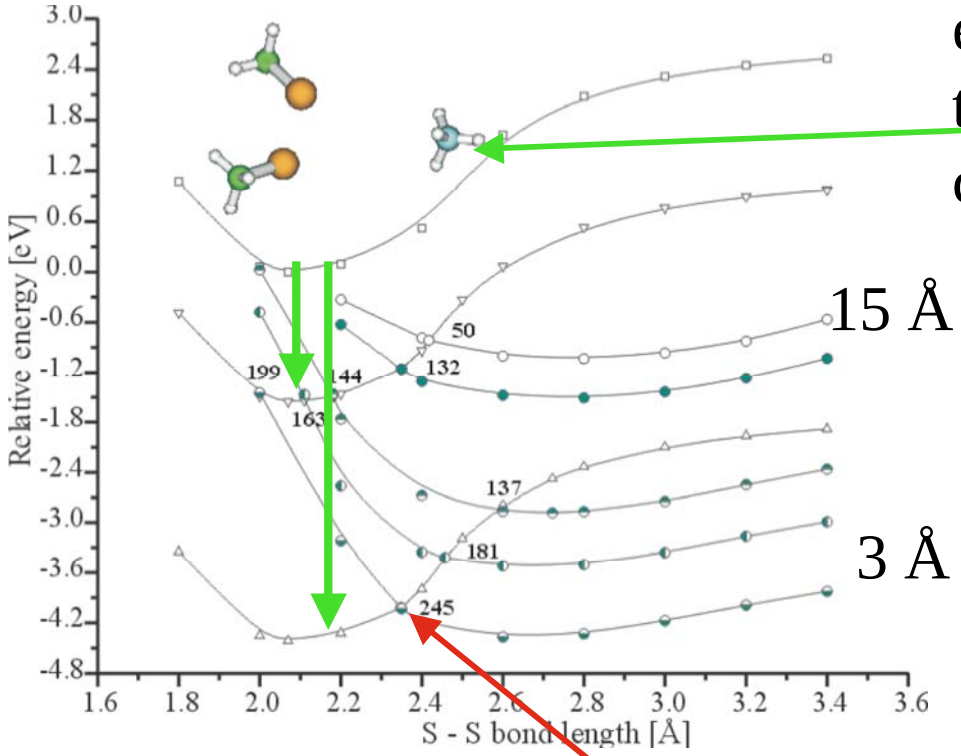
Recently, McLuckey studied S-S cleavage using ETD on peptides containing protonated sites and fixed-charge sites





Their data suggested that transfer from charged sites to the S-S bond or among charged sites may occur.

We used Landau-Zener theory to estimate the rates of through-space transfer from protonated or fixed-charge sites to S-S bonds.

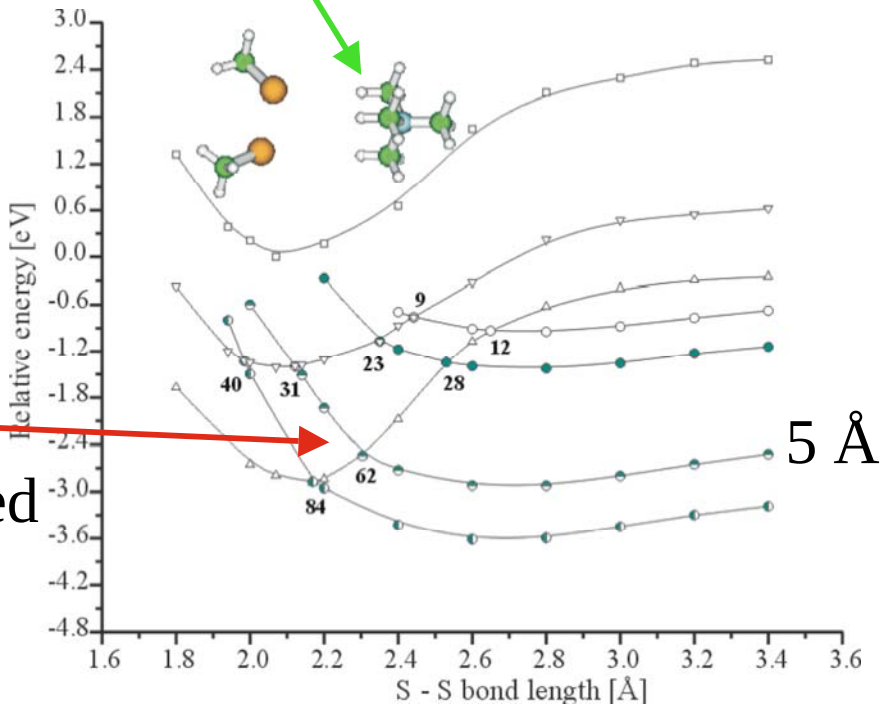


15 Å

3 Å

We found transfer from a protonated site to be feasible over ca. 3 Å and from a fixed-charge site over ca. 5Å

But we found transfer from protonated to fixed-charge sites to be slow.



5 Å

So, what do we conclude?

Electrons can attach either to + sites or, via Coulomb stabilization, to SS σ* or OCN π* sites. In the former case, the Cornell path can occur if the + site is within hydrogen bonding distance.
Subsequent cleavage of the SS σ bond is prompt;
cleavage of the N-C_α bond has a barrier of ca. 30 kcal mol^{-1}.

Electrons are more likely to attach at a + site, but then they can through-bond transfer only over ca. 4-5 bonds to end up on a π* or σ* site.

Electrons can attach to a + site and undergo through-space transfer to an S-S bond but only if the + site comes within 5 Å of the S-S bond.

Electrons attached to one + site are slow to transfer to another + site of different character.