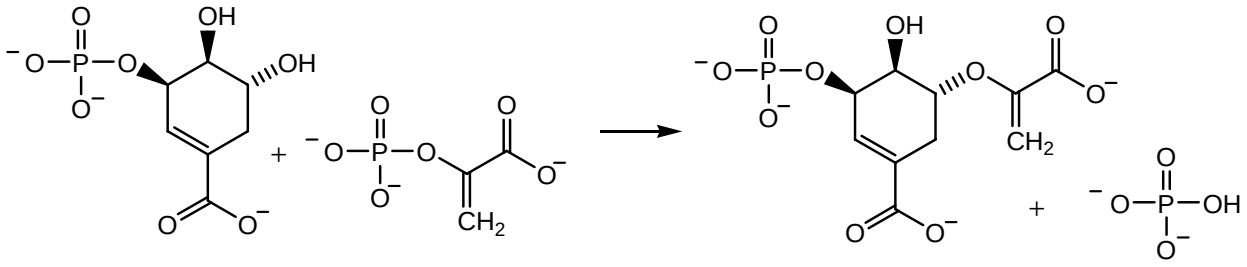
Chemistry 4011/8011 Wednesday, December 6

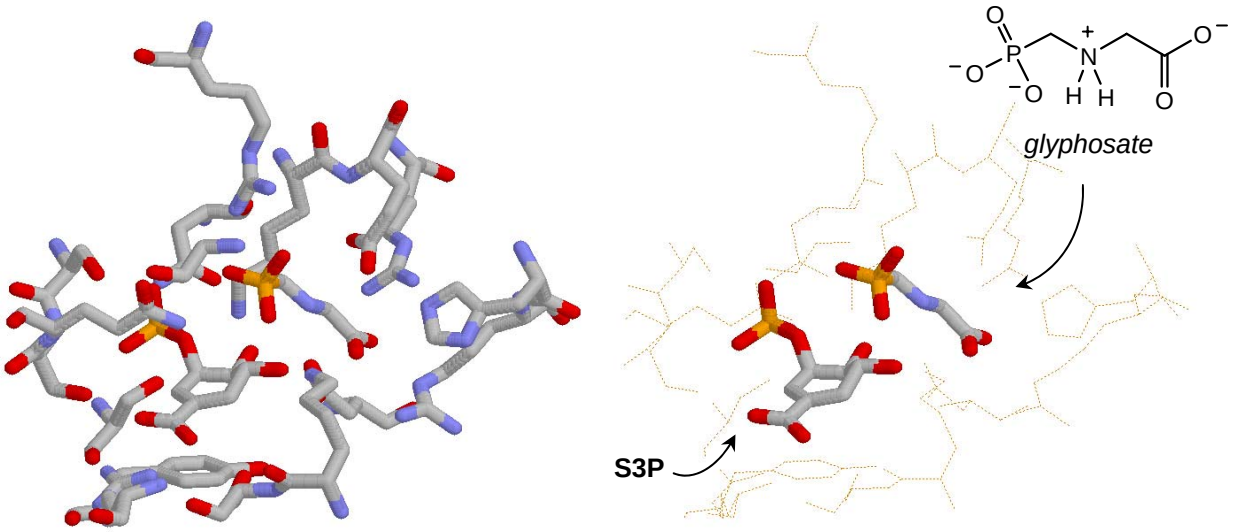
Workshop 11

The enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is part of a critical biosynthetic pathway in plants, and catalyzes the addition of phosphoenolpyruvate (PEP) to shikimate-3-phosphate (S3P):



S3P **PEP**

The catalytic activity of EPSPS can be inhibited by glyphosate (marketed as the herbicide Roundup® by Monsanto), which binds the enzyme active site along with S3P.[1] The complex of the EPSPS enzyme with S3P and glyphosate has been determined by X-ray crystallography;[2] two views of the active site are shown below:



(protein active site displayed) *(protein active site hidden)*

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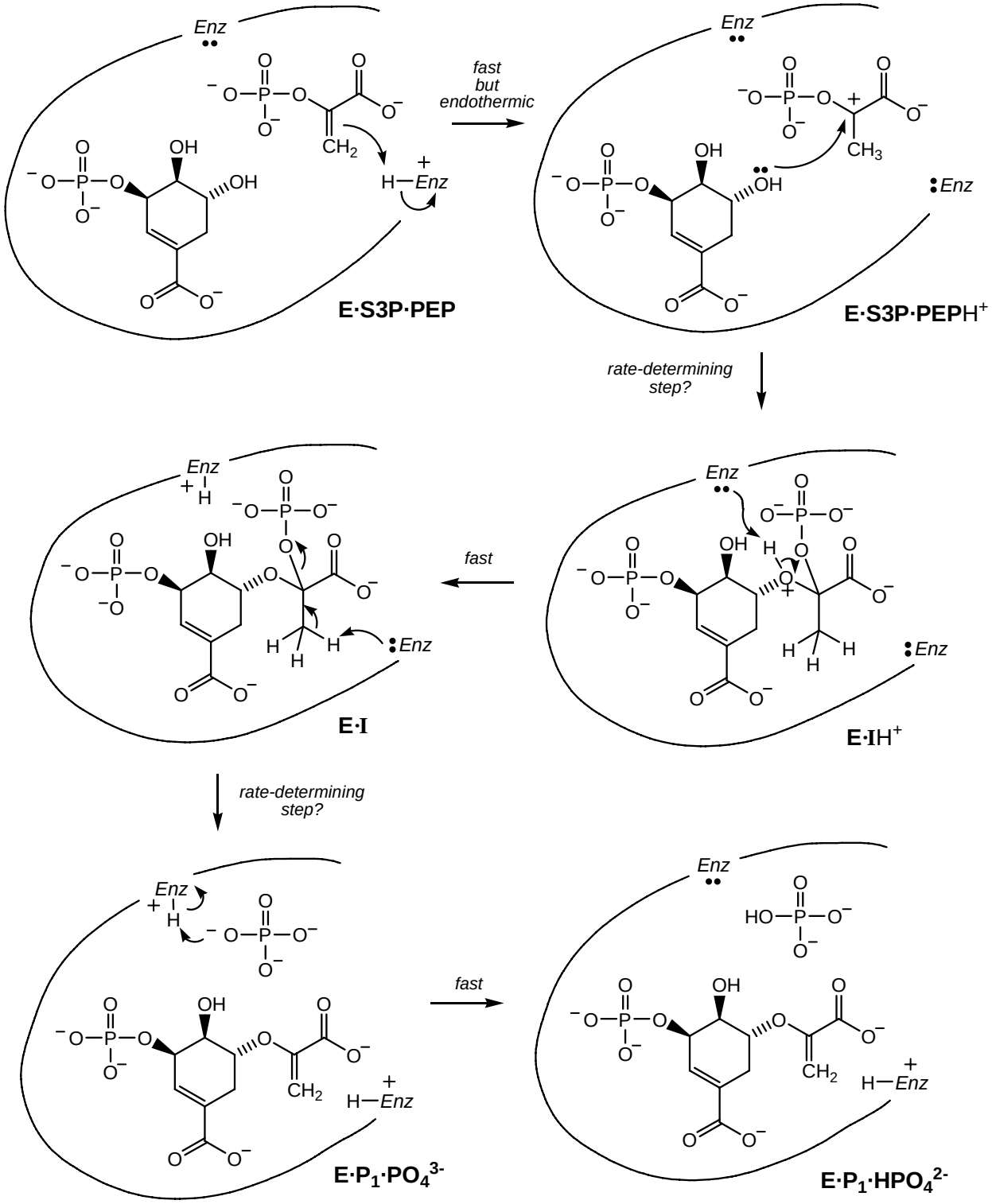
[1] Monsanto's success at marketing Roundup® as a broadly-applicable herbicide has been tempered by public reaction towards their development of Roundup Ready® canola, corn, cotton and soybeans, which have been genetically manipulated to express an enzyme that substitutes for EPSPS. Because they have this extra enzyme, Roundup Ready® plants survive when treated with glyphosate, while other (invader) plant species die.

[2] Schönbrunn, E.; Eschenburg, S.; Shuttleworth, W. A.; Schloss, J. V.; Amrhein, N.; Evans, J. N. S.; Kabsch, W. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 1376-1380.

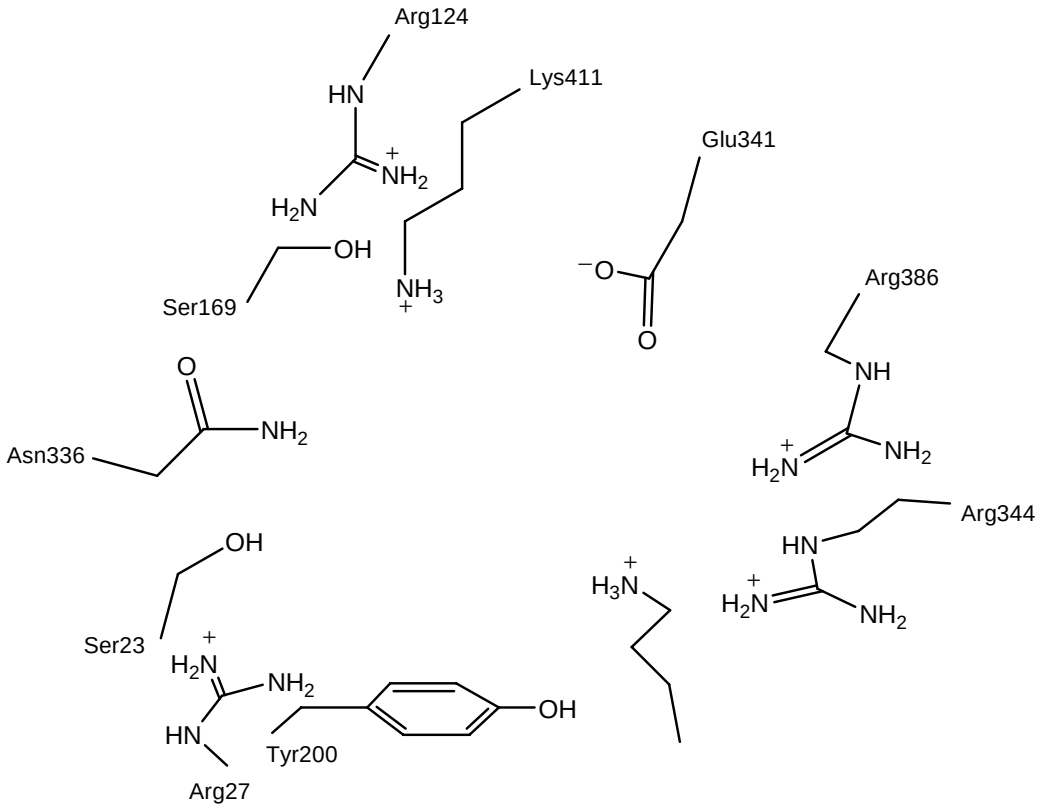
One proposed mechanism for the reaction between S3P and PEP involves sequential protonation, addition and elimination steps in the enzyme pocket:



a) Assume that glyphosate inhibits the enzyme so well because it mimics the rate-determining transition state of the reaction above. (In other words, assume that **E·S3P·glyphosate** resembles **E·TS**‡.) Which of the steps on the previous page is rate-determining, and what is the structure of its transition state?

b) Draw a potential energy diagram for the overall catalytic process. Label your diagram with $\Delta G^{‡}_{cat}$ (which determines k_{cat}).

c) Looking at the active site, do you think the catalyst is participating in favorable, enthalpic interactions in **E·TS**‡ that are not present in **E·S3P·PEP**? Or is the catalyst merely bringing **S3P** and **PEP** together? Draw the rate-determining transition state in the pocket below (reproduced from the first page, but without **S3P** or **PEP**) to illustrate your answer.



d) How would you expect $\Delta H^{‡}_{cat}$ and $\Delta S^{‡}_{cat}$ to differ from $\Delta H^{‡}_{uncat}$ and $\Delta S^{‡}_{uncat}$?

e) What kinetic experiments might you design to further test your hypothesis of which transition state is rate-limiting?

f) Monsanto researchers have found that goosegrass, a common weed, develops resistance to glyphosate over time due to mutations near the enzyme active site.[3] As a result, Monsanto is investigating other herbicides that have structures similar to that of glyphosate but that might bind these EPSPS mutants. How might EPSPS change in such a way that it would accept PEP and catalyze the reaction better than it binds glyphosate? What structural changes might Monsanto introduce to counter these mutations?

[3] Baerson, S. R.; Rodriguez, D. J.; Tran, M.; Feng, Y.; Biest, N. A.; Dill, G. M. *Plant Physiol.* **2002**, *129*, 1-11.