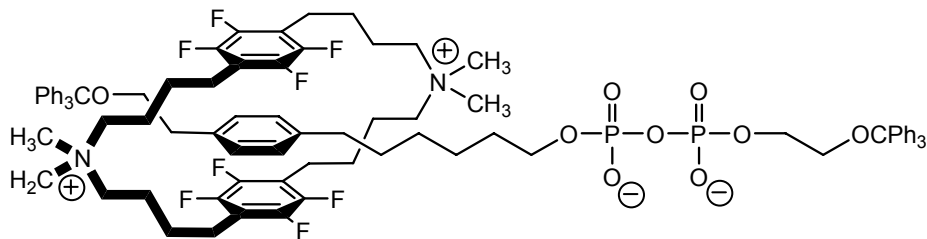
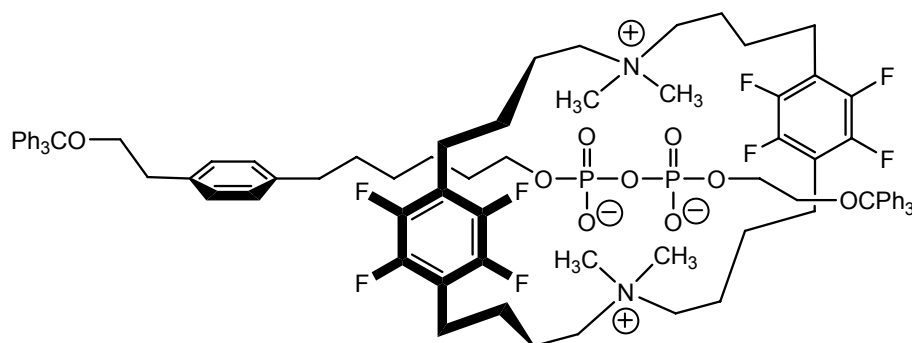


Problem Set 2 Answer Key

1. a) i) CHCl_3 is non-interactive—it has no H's to donate, and no lone pairs to use to accept H's, in H-bonds. As a result, the carbonyls of the macrocycle hydrogen-bond with the amide protons of the chain without competition. The macrocycle will be on the amide “station” of the chain.
- ii) An electron donor solvent such as DMSO has the ability to hydrogen-bond with the molecules. All amide N-H protons—both those on the macrocycle and those on the linear chain—will preferentially hydrogen-bond with the solvent over the macrocycle because the DMSO is effectively more concentrated than either macrocycle or chain. However, hydrophobic interactions between the alkane chain and the relatively hydrophobic ring will not be disturbed by the solvent, and the ring will spend most of its time on the alkane “station”.
- iii) An electron acceptor solvent will hydrogen bond with carbonyl lone-pairs on amides from both the chain and the macrocycle. Again, hydrophobic interactions between the alkane and macrocycle aren't affected. So, the result has the macrocycle “stationed” on the alkane end.
- b) There are many possible answers to this question. The question asked that you not use interactions from Leigh's system—so no hydrogen bonding or hydrophobic effect—but Anslyn and Dougherty outline a number of other possible interactions in Chapter 3 you could use here. One example would be a system that uses some kind of arene-arene interaction (*MPOC* p. 184) as one interaction element, and electrostatic salt-bridging (*MPOC* p. 165) as the other:





In this example, the macrocycle would be on the first, arene-stacked station in the presence of a solvent that would screen the interaction of salt bridges—like high-dielectric H₂O, for example, which *MPOC* explains weakens the effect of salt bridges on biomolecule surfaces. The macrocycle would be at the second station, on the other hand, in a solvent that would compete for the arene-arene stacking interaction—like in benzene, for example, which would itself interact with the electron-deficient fluorine-substituted aromatic rings.

This is, of course, just an example. You could make up lots of different structures to answer this problem.

2. a) The amino acid substitutions in the binding pocket where K_{rel} is larger than 2 had effects on binding the respective substrates. In these substitutions, the substrates are bound less tightly.

$$K_{rel} = K_{d, substituted} / K_{d, unsubstituted}$$

$$\text{and } K_d = [\text{enzyme}][\text{ligand}] / [\text{enzyme-ligand}]$$

Therefore, in the substituted case the fraction of unbound ligands is higher than in the unsubstituted case, thus substrates are bound less tightly for all substitutions. This makes pretty good sense when you look at the substitutions themselves—in each case, the substitution has replaced an H-bonding group with a non-H-bonding group. So we'd expect that each substitution would lead to weaker binding.

Tyr34 → Phe

$K_{rel, ATP} = 1.0$, there is no effect on binding ATP

$K_{rel, tyr} = 2.3$, there is a slight effect on binding tyrosine. The substrate will be bound ($\Delta G_{rel} = RT \ln K_{rel} = 1.99 \times 298 \times \ln(2.3)$) 0.5 kcal/mol less tightly.

Cys35 → Phe

$K_{\text{rel, ATP}} = 2.7$, here there is also a small effect on binding ATP. The binding energy will be ($\Delta G_{\text{rel}} = RT \ln K_{\text{rel}} = 1.99 \times 298 \times \ln(2.7)$) 0.6 kcal/mol less tightly.

$K_{\text{rel, tyr}} = 1.1$, this value is most likely within the experimental error and we can assume that there is no effect on binding tyrosine.

His48 → Gly

$K_{\text{rel, ATP}} = 1.2$ and $K_{\text{rel, tyr}} = 1.3$, again, these values are most likely within the experimental error, and we can assume there is no effect. (If you calculated, you'd get something absurdly small.)

Tyr169 → Phe

$K_{\text{rel, tyr}} = 1.0$, no effect

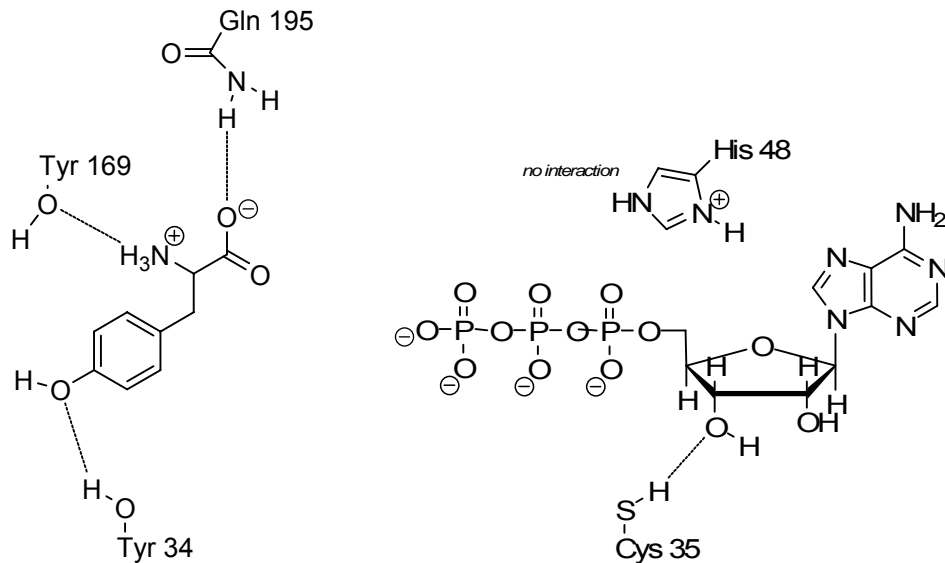
$K_{\text{rel, ATP}} = 460$, which is over two factors of 10 larger. The substrate will be bound over 2.8 kcal/mol less tightly. (Calculated from $\Delta G_{\text{rel}} = RT \ln K_{\text{rel}}$ gives a value of 3.6 kcal/mol)

Gln195 → Gly

$K_{\text{rel, tyr}} = 1.3$, this value is most likely within the experimental error.

$K_{\text{rel, ATP}} = 44$, which is over one factor of 10 larger. The substrate will be bound over 1.4 kcal/mol less tightly. (Calculated from $\Delta G_{\text{rel}} = RT \ln K_{\text{rel}}$ gives a value of 2.2 kcal/mol)

b)



c) The interactions calculated in part a) show a lower strength than the hydrogen bonds listed in *MPOC*. This would be due to competitive interactions such as hydrogen bonds with the solvent (water) and with other amino acids.

Interestingly, Fersht suggests that the interactions with very low but positive energy changes (Tyr34→Phe and Cys35→Phe) might have a water molecule in between the H-donor and acceptor in each case. If this were true, getting rid of an H-bonding amino acid would indeed reduce the affinity of the substrate for the pocket, but maybe the intervening water molecule could still stabilize the ligand in there a little, making it not quite so bad.