# Chapter 3 Odd Solutions

**1.** This question can of course be answered in great detail by drawing detailed structural diagrams. However, the essentials are fairly simple, as suggested by the question. (a) The enzyme is a tetramer of four identical subunits. (b) On activation, one dimer rotates relative to the other by about 7°, in a manner very reminiscent of hemoglobin. In the paper, the authors have a detailed discussion of water molecules in the interface that is not very relevant to this answer (though it is of relevance to Chapter 6). (c) In the R state, the fructose 6-phosphate (Fru6P) substrate has a salt bridge from its phosphate to Arg 162, which is suggested to lead to strong binding. In the T state, the rotation of one dimer relative to another leads to a change in conformation of the loop (6-F loop, residues 155–162; Figure 4 in the paper), which leads to a 180° flip of Arg 162 and Glu 161, such that Glu 161, which was pointing away from the Fru6P-binding site in the R state, now points in toward it, while Arg 162 now points away. This is indicated by Figure 3 in the paper, but can be shown much more clearly by a modern graphics system. An ideal answer would include a diagram that shows the 6-F loop backbone, highlighting the side chains of Glu 161 and Arg 162 in the two states, with the position of Fru6P in the R state. The consequence is that the positively charged Arg 162 side chain is now replaced by a negatively charged Glu 161 side chain, and hence the binding of Fru6P is greatly weakened. It is also unable to bind in the catalytically active conformation.

It is worth noting that the T state can only be formed when the ADP effector site is blocked by the binding of PEP. This cannot happen when the larger ADP is bound.

In the final section of the paper, the authors note that any intermediate state would be unstable, demonstrating why the allosteric transition must be concerted. A very nice account of PFK function is given by C. Branden and J. Tooze [Introduction to Protein Structure, 2nd ed. New York: Garland, 1999, pages 114–117].

**3.** This question has been investigated by S.A. Teichmann, for example in E.D. Levy et al. [*Nature* 453:1262–1265, 2008] and also discussed in A.J. Venkatakrishnan, E.D. Levy and S.A. Teichmann [*Biochem. Soc. Trans.* 38:879–882, 2010]. The answer is that quaternary structure is conserved almost as well as tertiary structure, and is therefore better than function. If two sequences are 20–30% identical, their quaternary structures will be the same in roughly 60% of cases. See also J. Janin, R.P. Bahadur and P. Chakrabarti [*Quart. Rev. Biophys.* 41:133-180, 2008].

**5.** Fos (UniParc P01100; http://www.uniprot.org/uniprot/P01100?conversationContext=1): starts at residue 139 (start of basic sequence). Basic residues are in red, leucines at position d of the heptad repeat are in blue. The three sequences are the basic region, linker, and leucine zipper region.

KRRIRRERNKMAAAKCRNRRRE LTDT LQAETDQLEDEKSALQTEIANLLKEKEKL

Jun (UniParc P05412; http://www.uniprot.org/uniprot/P05412?conversationContext=3): starts at residue 257 (start of basic sequence). Colors are as for Fos.

RKRMRNRIAASKCRKRKLER IAR LEEKVKTLKAQNSELASTANMLREQVAQL

The acidic glutamates of Fos are in green, and the basic lysine and arginine of Jun are in magenta. The acidic residues are at position g of the heptad, and the basic residues are at position e, which are approximately opposite each other.

**N1.** Yes. The expected number of cuts is $4.72 \times 10^6 \times 4^{-6} = 1152$. The number of cut sites here can be described roughly as a normal distribution with a mean of 762 and a standard deviation of 494, in which case the value 1152 falls well within one standard deviation above the mean. For a more rigorous test one could perform for example a single sample *t*-test, which would return a *t* statistic of 2.23, in contrast with a critical value of 2.36 for seven degrees of freedom, again implying that the mean of the sample is not significantly different from the expected mean. The paper cited discusses evidence that the locations of cut sites for the enzymes seem to be randomly distributed, except possibly for *Bam*HI sites, which seem to be more evenly spaced than expected.

**N3.** $\Delta G = -RT \ln K$. Here $\Delta G = -50$ kJ mol$^{-1}$ or $-5 \times 10^4$ J mol$^{-1}$, $T = 298$ K and $R = 8.31$ J K$^{-1}$ mol$^{-1}$. Therefore $\ln K = \Delta G/RT = 5 \times 10^4/(298 \times 8.31) = 20.19$, or $K = 5.9 \times 10^8$.