I have put an XPP program on my website for a type 1 coherent FFL with AND logic. I encourage you to play around with this to get the feel for what this motif can do and how its behavior varies with changes in parameters. Some explanation is below, along with ideas for some things you can look at.

Some explanation of the program
It is called C1_FFL_AND.ode, and is basically what is described on page 47 of Alon. A few things to notice in the program:

- Three curves are plotted together, X, Y, and Z. This is achieved in the line of code beginning with the XPP parameter nplot=3. You could achieve the same thing by running XPP and then in the main window clicking “Graphics Stuff” and then “Add” (or type ga). Then put whatever you want (like Y) on the Y-axis and set Color to some integer other than 0 (which is black). After clicking OK you will see that a second curve has appeared on the screen. You could do it again to plot the Z time course. To delete a curve, click “Graphics Stuff” and then “Delete” (or type gd).

- There are three switches coded into the program. The first indicates when signaling molecule $S_x$ is initially turned on ($tonx1$) and then turned off ($toffx1$). The second indicates when this happens again, later in time ($tonx2$ and $toffx2$). The third indicates when signaling molecule $S_y$ is turned on and off ($tony$ and $toffy$).

- The parameters set the production rates ($\beta$), degradation/dilution rates ($\alpha$), and affinities ($K$). These are all things you can vary, providing hours of entertainment.

- Notice how I defined the AND gate. This uses a Heaviside function, and says that each input must be greater than 0.9 for the function to equal 1 (ON). Otherwise it is 0 (OFF).

- I have defined the affinities as auxiliary functions. This allows you to plot them. You can superimpose one or more of these on your plots of X, Y, or Z to see what happens when a threshold is reached. For example, if you plot X, Y, and Kxy on the same graph, then, you will see that Y starts to increase when X goes past the horizontal Kxy line.

Exercises
The work below should be turned in. Please print out relevant figures and label completely. Also, take care in describing the figures.

(1) Change the on/off times of $S_y$ so that only one response in $Z$ occurs. Then, starting with the original parameter values, change the duration of one of the pulses of $S_x$ so that only one $Z$ response occurs. Then, starting with the original parameter values, change the threshold for $Y$ binding to its $Z$-promoter so that $Z$ never responds to the $S_x$ pulses. Finally, adjust the $Y$ degradation rate so that, in this last case, $Z$ does respond to both $S_x$ pulses.

(2) Write a code for a C1-FFL-OR motif (turn in a copy of the code). The changes from C1-FFL-AND are very minor, but the behavior of the system is very different. Verify that this exhibits a delayed OFF response, but no delay in the ON response.

(3) The regulator $Y$ in C1-FFLs in transcription networks is often negatively autoregulated. How does this affect the dynamics of the circuit, assuming that it has an AND input function at the $Z$ promoter? How does it affect the delay times? The $Y$ regulator in an OR gate C1-FFL is often positively autoregulated. How does this affect the dynamics of the circuit? How does it affect the delay times?

(4) Code up the type-3 coherent FFL (see slides showing 8 possible FFLs) with AND logic at the $Z$ promoter in response to steps of $S_x$ (turn in a copy of the code). Here, AND logic means that $Z$ is produced if both $X^*$ and $Y^*$ do not bind the promoter. Are there delays? What is the steady-state logic carried out by this circuit?

(5) Write a code for a type 1 incoherent FFL (turn in a copy of the code). Consider a case where $X$ in the I1-FFL begins to be produced at time $t = 0$, so that the level of protein $X$ gradually increases. The input signals $S_x$ and $S_y$ are present throughout. Analyze a set of genes $Z_1$, $Z_2$, and $Z_3$, all regulated by the same $X$ and $Y$ in the FFL. Design thresholds such that the genes are turned ON in the rising phase in a certain temporal order and turned OFF in the declining phase of the pulse with the same order. Next, design thresholds such that the turn-OFF order is opposite to the turn-ON order. Plot the resulting dynamics and give threshold values used.