I'm trying to work out the numerical solution to a ligand binding competition experiment scenario.
The situation is the following: I form a complex of Protein ( P ) and Ligand (L), whose affinity to each other is defined by the dissociation constant $K d_{\text {PL }}$ (Eqn 5). The experiment is to determine this very constant, $K d_{P L}$.
The ligand is the observable quantity (fluorescent) and it fluoresces more when it is in the complex. So $\mathrm{F}(\mathrm{L}) \ll \mathrm{F}(\mathrm{PL})$. This is the starting scenario: a solution mainly composed of [PL]. This complex [PL] is made by mixing two known quantities (concentrations) of $\mathrm{P}_{0}$ and $\mathrm{L}_{0}$. Into this a competitor C is titrated (of known concentration), which competes with fluorescent L for P . As more and more C is added, the less [PL] remains, and the more [PC] forms.

However it is more complex than this: the formation of PC is subject to its own affinity (dissociation) constant $\mathrm{Kd}_{\mathrm{PC}}$, as defined by Eqn 6. The value of $\mathrm{Kd}_{\mathrm{PC}}$ is known.

L and $[\mathrm{PL}]$ are the observed quantities, so I get a curve resembling an exponential decay, as [PL] is gradually reduced to form [PC] and free L (the latter fluorescing less).

The observed quantity $\mathrm{F}_{\text {obs }}$ is thus described by Eqn $7, \mathrm{~F}_{\mathrm{L}}$ and $\mathrm{F}_{\mathrm{PL}}$ are the fluorescence values for free L and complex [PL].

Essentially I have to find a way to mathematically describe the concentrations of the free concentrations of [L] and [PL].

The overall reaction equation is described by Eqn 1.
Then there are the mass equations:
The total (known) amounts of P, C and L are described by Eqns 2, 3, 4, respectively.
Competition equation
Definitions:
$\mathrm{P}=$ Protein
L = Ligand, fluorescent, observable
$\mathrm{C}=$ Competitor, which is similar to L but non-fluorescent
$\mathrm{PL}=$ Protein ligand complex (fluorescent, observable)
$\mathrm{PC}=$ Protein competitor complex
$\mathrm{P}_{0}, \mathrm{C}_{0}, \mathrm{~L}_{0}=$ Total protein, competitor and ligand concentrations, respectively
$\mathrm{F}_{\text {obs }}=$ observed fluorescence
Reaction equations (during the titration experiment)

1. $[\mathrm{PL}]+\mathrm{P}+\mathrm{L}+\mathrm{C} \rightarrow[\mathrm{PC}]+[\mathrm{PL}]+\mathrm{P}+\mathrm{L}+\mathrm{C}$ At the beginning of the experiment $\mathrm{C}=0$

Mass conservation equations:
2. $\mathrm{P}_{0}=[\mathrm{PL}]+\mathrm{P}+[\mathrm{PC}]$
3. $\mathrm{C}_{0}=[\mathrm{PC}]+\mathrm{C}$
4. $\mathrm{L}_{0}=[\mathrm{PL}]+\mathrm{L}$

Equilibrium Equations:
5. $K d_{P L}=\frac{[P][L]}{[P L]}$
6. $K d_{P C}=\frac{[P][C]}{[P C]}$

Finally
7. $\mathrm{F}_{\mathrm{obs}}=\mathrm{F}_{\mathrm{L}}[\mathrm{L}]+{ }_{\mathrm{F}}[\mathrm{PL}]$

To summarise:

The known quantities are $\mathrm{P}_{0}, \mathrm{C}_{0}, \mathrm{~L}_{0}$, as well as the Kd of the competitor, $\mathrm{Kd}_{\mathrm{PC}}$
The observed quantity is L and [PL] as $\mathrm{F}_{\text {obs, }}$, but I do not know their concentrations. These I have to define mathematically (see eqn 7)

Time to get serious:
As $\mathrm{L}=\mathrm{L}_{0}-[\mathrm{PL}]$ (rearrangement of eqn 4), I 'simply' have to find a way to describe [PL] as Eqn 7 then is
8. $\mathrm{F}_{\text {obs }}=\mathrm{F}_{\mathrm{L}}\left(\mathrm{L}_{0}-[\mathrm{PL}]\right)+\mathrm{F}_{\mathrm{PL}}[\mathrm{PL}]$

The value of $\mathrm{F}_{\mathrm{L}} / \mathrm{F}_{\mathrm{PL}}$ arise from the curve fit, they don't have to be defined.
I start substituting into eqn 5 using 2 :
9. $K d_{P L}=\frac{(P o-[P L]-[P C])(L o-[P L])}{[P L]}$

Substituting into 6 using 2, 3:
10. $K d_{P C}=\frac{(P o-[P L]-[P C])[\mathrm{Co}-[P C]]}{[P C]}$

All good so far, because $\mathrm{P}_{0}, \mathrm{C}_{0}$, are known and I can solve 10 for [PC ] and plug it into 9 .

I can solve 9. using the quadratic equation for [PL], first to $a x^{2}+b x+c$ :
$0=$ PoLo - Po $[P L]-[P L] L o+[P L]^{2}-[P C] L o+[P C][P L]-[P L] K d_{P L}$
11. $0=[P L]^{2}+[P L]\left(-P o-L o+[P C]-K d_{P L}\right)+$ PoLo $-[P C] L o$
$x=\frac{-b \pm \sqrt{b^{2}-4 a c}}{2 a}$,
12. $x_{P L 1,2}=\frac{-\left(-P o-L o+[P C]-K d_{P L}\right) \pm \sqrt{\left(P o+L o-[P C]+K d_{P L}\right)^{2}+4(L o(P o+[P C]))}}{2}$

This is all good, but unfortunately there's the term [PC]!!!!!
I can solve equation 10 for PC , too:
$0=\mathrm{PoCo}-\mathrm{Po}[P C]-[P L] \mathrm{Co}+[\mathrm{PL}][P C]-[P C] C o+[P C]^{2}-[P C] K d_{P C}$
13. $0=[P C]^{2}+[P C]\left([P L]-\mathrm{Po}-\mathrm{Co}-K d_{P C}\right)+\mathrm{PoCo}-[P L] \mathrm{Co}$

From this I can get the quadratic solution again.
14. $x_{P C 1,2}=\frac{-\left([P L]-P o-C o-K d_{P C}\right) \pm \sqrt{\left([P L]-P o-C o-K d_{P C}\right)^{2}-4(P o C o-[P L] C o)}}{2}$

Now, I'd have to substitute $x_{P C l, 2}$ from eqn 14. into eqn 12 or 11, and solve this for [PL] one more time.
How??
Substitution:
Simplification of 14:
15. $x_{P C 1,2}=\frac{-(n+[P L]) \pm \sqrt{(n+[P L])^{2}-s t+s[P L]}}{2}$

Where $\mathrm{n}=-\mathrm{Po}-\mathrm{Co}-\mathrm{Kd}_{\mathrm{PC}}$
Where $\mathrm{s}=4 \mathrm{Co}$
And $t=P o$

This needs to be substituted into 11:
Simplifying 11:
16. $0=[P L]^{2}+[P L](a+[P C])+b c-[P C] c$
where $\mathrm{a}=\left(-\mathrm{Po}-\mathrm{Lo}-\mathrm{Kd}_{\mathrm{PL}}\right)$
and where $\mathrm{b}=\mathrm{Po}$
and $\mathrm{c}=$ Lo

Substituting 15 into 16)
Eqn 17.:
$0=[P L]^{2}+[P L]\left(a+\left[\frac{-(n+[P L]) \pm \sqrt{(n+[P L])^{2}-s t+s[P L]}}{2}\right]\right)+\left(b c-\left[\frac{-(n+[P L]) \pm \sqrt{(n+[P L])^{2}-s t+s[P L]}}{2}\right] c\right)$
How do I solve this for [PL]?

