By analyzing data from molecular dynamics simulations at room temperature, the authors study the fluctuations of crambin, a small globular protein, around its native state. They focus especially on the corrections to the simplest armonic description of protein fluctuations and analyze in detail the contribution from anaharmonicity and the coupling of differente modes.

The paper is well written, the arguments/computations convincing and the result interesting: at room temperature around 6% of the whole fluctuation entropy of a protein around its native state is taken into account by non-harmonic contribution. I find the paper suitable for publication in Physics Biology.

I have a couple of comments the authors might like to consider

1) The authors define initially fluctuations as deviations from equilibrium positions, but then use in practice average atom position in the simulation as an estimate of the former. I would argue that the latter is in fact the correct definition, as it makes possible the analysis of "double well" cases, such as the one found for crambin (see fig. 3). Indeed, in case of asymmetric wells, average and equilibrium position do not correspond.

2) The authors find that higher order computation of non-harmonic corrections increases the fluctuation entropy. Is this a general rule? Could this be related to the "double well" feature of the slowest mode?

3) The eigenvalues of the covariance matrix are just mentioned and never appear in any equation. It could be useful for the general reader to show how they actually enter the game (e.g. in the evaluation of entropy for the pure harmonic case). Moreover: could one state that mode coupling is relevant for mode pairs chosen among the slowest modes?