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Evaluation of Ph.D. thesis by Klára Grantz Šašková

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The thesis by Klára Grantz Šašková entitled "HIV-1 Protease: Insights into Drug Resistance Development" consists of a 57 page review of the HIV pandemic, the structure and function of HIV-1 protease, inhibitor design and development of drug resistance followed by an overview of the four major publications that make up the core of her thesis. These four manuscripts include two first author publications in 2008 and 2009 in *Protein Science* and *Journal of Virology*, respectively; and a third authorship paper in 2007 and second authorship in 2009 in *Journal of Molecular Biology* and *Journal of Virology*, respectively. In addition, during the time period of her thesis (2008-2009), Ms. Šašková was also a contributing middle author on four other manuscripts also primarily involving HIV-1 protease: two in *Journal of Medicinal Chemistry, Biomolecular NMR Assignments* and *Antiviral Research*. Combined this is a very impressive body of work and all of the journals are highly respectable, internationally renowned journals.

Four aims related to understanding molecular basis for drug resistance in HIV-1 protease were successfully addressed as part of this thesis: (1) Evolution, structure and thermodynamics of Nelfinavir resistance; (2) Study of the Lopinavir associated flap mutation I54A in isolation and with other resistance associated mutations; (3) The impact of unusual insertions into HIV-1 protease on the enzyme's structure and function and (4) The structure and activity of highly mutated HIV-1 protease variants isolated from the patients failing darunavir therapy. While all four aspects of the thesis contribute to the scientific communities understanding of drug

resistance in HIV-1 protease. The fourth aim is quite an achievement as these variants are often very difficult to work with biochemically. In addition these patient derived Darunavir resistant variants are particularly significant and relevant for the drug design community as these variants give the first glimpse into why the highly potent latest generation HIV-1 protease inhibitor, Darunavir, may fail. The resulting structures will likely aid future structure based drug design efforts.

Formal questions:

1) Some argue there is no longer a need for additional HIV protease inhibitors. Why do they think this? Why would you argue otherwise?
2) Generally, do you think that insertions in HIV protease may be a result of preserving the RNA structure of HIV along the lines of the Weeks paper *Nature* 2009 460(7256):696-8, how about other patterns of resistance?
3) When looking at structural changes, whether RMSD, van der Waal contact or hydrogen bond, between complexes of HIV protease, how do you assess what a significant change is and why?

Specific questions and remarks – Please note these <u>do not</u> need to be specifically addressed as part of the defence, but are for the future reference of the defendant.

1) D30N and L90M do occur together if N88D is present RW Shafer paper *AIDS RES. AND HUMAN RETRO.* 2006 22(12):1300–1305
2) In discussion of co-evolution and impact of changes in the cleavage sites, should include work presented in Kolli M, et al. *Virology*. 2006 347(2):405-9 and Kolli M, et al. *J Virol.* 2009 83(21):11027-42 where co-evolution was seen with increased levels of resistance.
3) Use of "catalytic triad" for aspartyl protease, although sometimes used, is a bit unwarranted, as only the Asp is involved in the catalysis; the other two residues are more conserved for geometrical reasons.
4) ATV (not NFV) is the only currently used protease inhibitor that is used unboosted.
5) In scientific writing the use of the word "it" needs to be minimized, redundancy is better than a loss of clarity by the overuse of "it". Also words like "Nowadays" is essentially slang and shouldn't be used.

In conclusion, in my opinion as a biophysicist and structural biologist with expertise in the molecular basis for drug resistance in HIV-1 protease, the thesis entitled “HIV-1 Protease: Insights into Drug Resistance Development” represents an impressive body of excellent research that is well deserving of the scientific degree of Doctor of Philosophy (Ph.D.) to be conferred to Klára Grantz Šašková.

Sincerely,

Celia A. Schiffer, Ph.D.
Professor of Biochemistry and Molecular Pharmacology