ERRATA

The corrections of the typing errors, word duplications, missing words or incorrectly reffered numbers of chapters and figures in text of the thesis are **highlighted**:

p. 8: "Pro studium mechanismu vazby darunaviru na mutovanou HIV-1 proteasu **bylo zvoleno** několik přístupů, např. jaderná magnetická rezonance (NMR), povrchová plasmonová rezonance (SPR) či kinetická měření využívající spektrofotometrické metody."

p. 9: APS- **ammonium** persulphate (instead of amonium)

p. 10: NtRTI- **nucleoside** reverse transcriptase inhibitor (instead of nucleotide)

p. 11: *pol*- gene encoding for viral **enzymes** (instead of enyzmes)

p. 44: „Determination of the stability **and** enzymatic activity of tag-extended variants"

p. 47: In chatper 4.3.: **Enzymes** (instead of Enyzmes)

p. 50: TAE buffer (**1x**) – The storage buffer was used and it was not diluted. This sign (number x) means dilution in the final volume, normally.

p. 51: „Cells were incubated with 850 μl of sterile **LB** medium at 37°C for one hour."

p. 52: „The concentration was deterimined by measuring the absorbance at **260 and** 280 nm using Nanodrop® ND-1000 Spectrophotometer (Thermo Scientific, USA)."

p. 56: The number of the chapter in text is reffered incorrectly:
„The isolation and purification procedures of the inclusion bodies were monitored by SDS PAGE (**chapter 5.4.2.**)." (instead of 4.4.2)

p. 57: „HIV-1 protease was then refolded by rapid **dilution** into 25-fold volume excess of water." (instead of dilusion)

p.58: „Ni-NTA resin was washed and equilibrated with **15** ml of solution A."

„Isolated inclusion bodies, containing denatured His-tagged HIV-1 protease were solubilized in 80 ml of **solution A**." (instead of solution AB)

p. 60: The numbers of chapters in text are reffered incorrectly:
„The progress of purification was analysed by SDS-PAGE (**chapter 5.4.2**.) and by western blotting (**chapter 5.4.5**.)." (instead of 4.4.2 and 4.5.4)

p. 61: „The analysis of the sample protein composition was carried out by vertical protein **electrophoresis** (Amersham Biosciences)."

p. 65: Comment to the concentrations of substrate and inhibitor, which were chosen for determination of the inhibition mode: Inhibitor concentrations were chosen considering the value of the IC50 for darunavir and mutated protease (MUT PR). Although, it was not measured, IC50 value can be easily calculated: Ki = IC50/(1+S/Km), when K_i= 1.2 nM reffers to the inhibitory costant for darunavir

inhibition of mutated protease (K_m= 141 µM). Substrate concentrations were chosen considering the value of Michaelis constant for mutated protease MUT PR.

p. 66: Figure 11: the mutation L10I is missing in the picture showing MUT PR and inMUT PR and it has to be considered as well.

p. 77: Figure in text is reffered incorrectly: **Fig. 21b (**instead of Fig. 20b)

p. 79: Figures in the text in chapter 6.4.3.2 are reffered incorrectly:
„SDS-PAGE as well as western blots in **Fig. 21b** show that some degradation products occurred during cation exchange purification. Their identity was analysed by protein microsequencing (for results see chapter 6.6.2.). Despite of the presence degradation product about 2-3 kDa, F1 fraction **(Fig. 21b, lane 4)** was further used for kinetic characterization.“ (instead of Fig. 5 and Fig. 20b, lane 4, respectively)
„Cell lysis supernatant contained only about 10% of biotinylated (22b, **lane 6**).“ (instead of lane 5)

p.87: The Figure 27 description is without following sentences:
„**Chyba! Záložka není definována.Chyba! Záložka není definována.“**

p. 90: „The yield of purified active enzyme was slightly lower in comparison to that of the **inactive** one.“ (instead of active)

„**Negative** effect of D25N mutation on the refolding process of inMUT variant and the auto-degradation of MUT protease variant during measurement may serve as a likely explanation.“ (instead of sNegative)

p. 94: Using the spectrophotometric assay, the inhibitory mechanism for darunavir and mutated protease was interpreted as that of the mixed type, **which is consistent with** an alternative binding site for the inhibitor outside the PR active site. (instead of duplication of that part)