APPLIED MICROBIOLOGY AND BIOTECHNOLOGY

SUPPLEMENTARY MATERIAL

Immunoprophylactic properties of the *Corynebacterium pseudotuberculosis*-derived MBP:PLD:CP40 fusion protein

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Table S1. **Amino acid sequence of the MBP:PLD:CP40 fusion protein.** Highlighted: Maltose Binding Protein (grey), the cleavage site for TEV-protease (underlined in red), the PLD protein from *C. pseudotuberculosis* (green), the rigid linker (pink), the CP40 protein from *C. pseudotuberculosis* (blue), and the histidine tag (yellow).

Amino acid sequence
MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDG PDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALS LIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYE NGKYDIKDVGVDNAGAKAGLTFLVDLKNKHMNADTDYSIAEAAFNKGETAMTINGP WAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLT DEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYA VRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGIEGRGGENLYFQSMASP ASTANRPVYAIAARVLTTQGVDDAVAIGANALEIDFTAWGRGWWADADGIPTSAGA TAEEIFKHIADKRKQGANITFTWLDIKNPDYCRDARSVCSINALRDLARKYLEPAGVR VLYGFYKTVGGPAWKTITADLRDGEAVALSGPAQDVLNDFARSENKILTKQKIADYG YYNINQGFGNCYGTWNRTCDQLRKSSEARDQGKLGKTFGWTIATGQDARVNDLLGK ANVDGLIFGFKITHFYRHADTENSFKAIKRWVDKHSATHHLATVADNPWEAAAKEA AAKEAAAKEPADLSQAPLKASPGHADKVGVQTTCDAKPIFFGYYRTWRDKAIQLKD DDPWKDKLQVKLTDIPEHVNMVSLFHVEDNQKSDDQFWETFRKEYQPKLKERGTRV VRTVGAQLLLNKIKEKGLYGRSVEDDYKYREIARDIYKKYVTDHNLAGLAVDMALR KVEKRIDLQWQLRKIMGAFSELMGPKAPANEGKKPGHEGYKYLIYDTFDNAQTSQV GLVADLVDYVLAQTYDKGTKESIDQVWNGFRDKINSCQFMAGYAQPEENDTNRFLT AVGEVNKSGAMQVAEWKPDNGVKGGTFAYALDRDGRTYDGDDFTTLKPTDFAFTK RAIELTTGESSTDLGKATGSRGHHHHHH

Table S2. Ratio between positive and negative ELISA optical density results. Serum samples from naturally infected or negative sheep and goats were tested in different dilutions in an immunoenzymatic assay, using the MBP:PLD:CP40 protein as antigen and in different concentrations. The results are expressed as means of three independent experiments.

Species	Antigen concentration (µg/mL)	Serum pool dilution			
		1:100	1:200	1:400	1:800
		Positive/Negative ratio			
Sheep	1	3.25	3.35	3.36	4.02
	2	3.23	3.97	4.08	1.98
	4	2.43	3.68	3.17	1.45
	8	2.04	2.17	2.80	3.25
Goat	1	2.48	2.33	3.16	2.86
	2	3.49	2.81	3.61	4.04
	4	2.50	4.29	2.68	1.98
	8	2.05	3.69	2.95	2.05

Table S3. The percentage of protein secondary structure fractions of the MBP:PLD:CP40 fusion protein through UV-CD experiments. Deconvolution and calculation of secondary structure percentages were performed by the CONTINLL software of the CDPro software package using the SP43 protein reference set. RMSD - root mean square deviation; NRMSD - normalized root-mean-square deviation.

Structure	α-helix	β-sheet	Turn	RMSD	NRMSD
%	19.6	30.4	20.8	0.016	0.016

Figure S1. 3D structure of the MBP:PLD:CP40 fusion protein. The structure in three dimensions was represented using the Chimera software. MBP is shown in grey, TEV protease site in red, PLD in green, the rigid linker in pink and CP40 in blue.

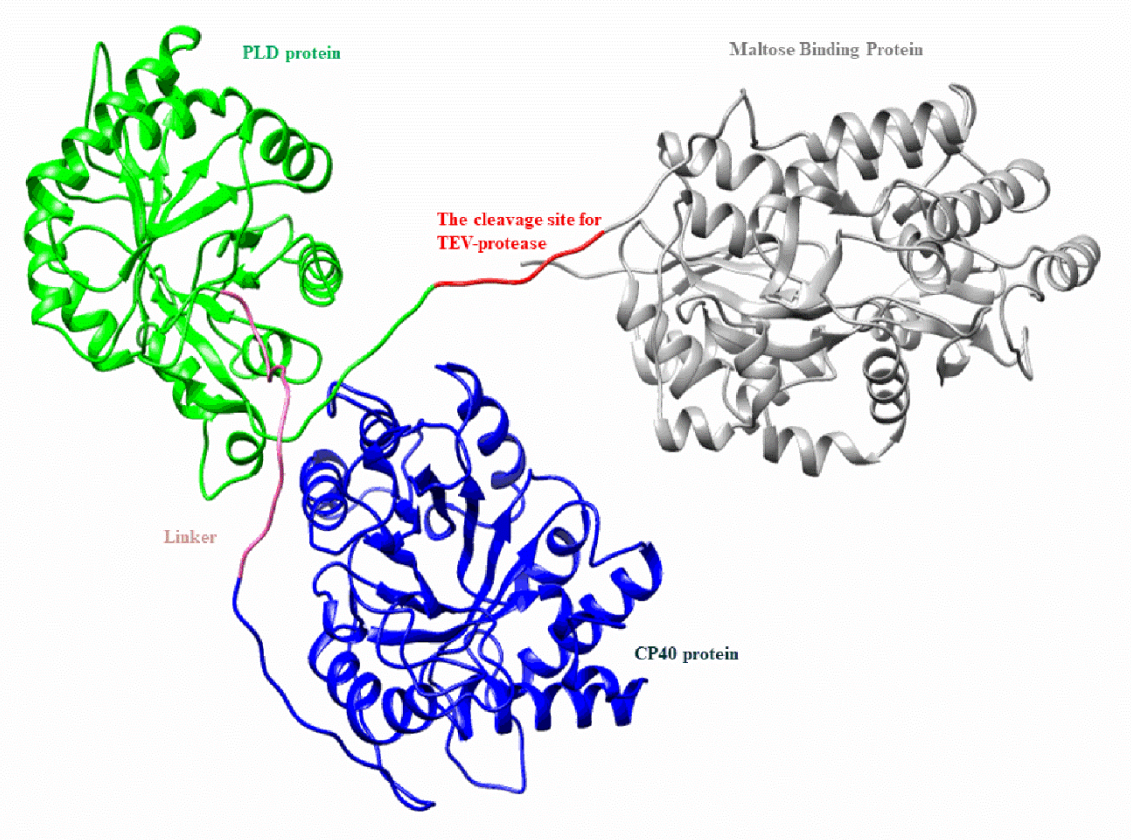

Figure S2. Analysis of the secondary structure of the proposed model for MBP:PLD:CP40 fusion protein using RaptorX software. The structure has 33% alpha-Helix, 16% beta-sheet and 50% coil.

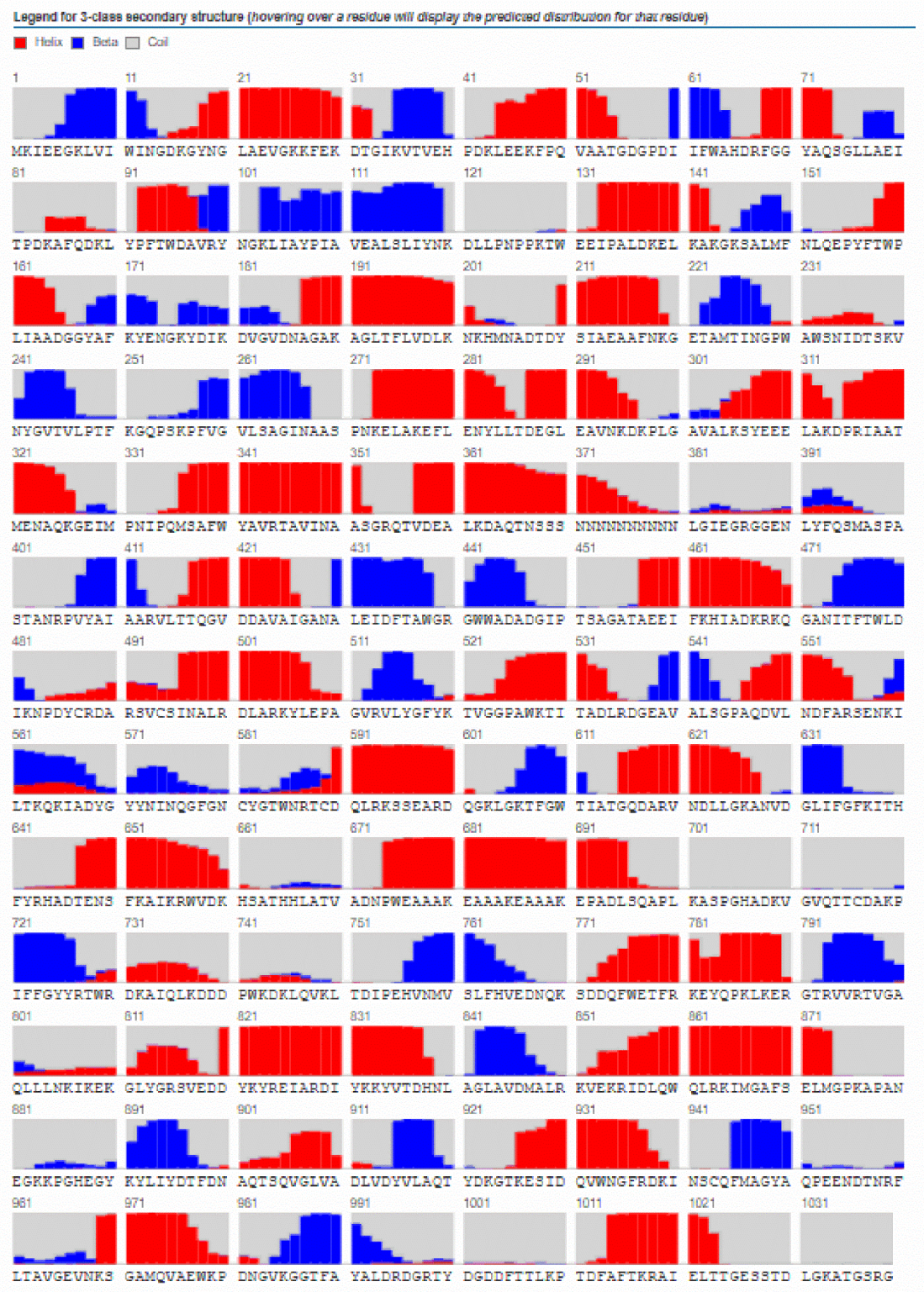

Figure S3. Ramachandran graph generated on the SAVE PROCHECK server for the analysis of the quality of the 3D structure of the MBP:PLD:CP40 fusion protein.

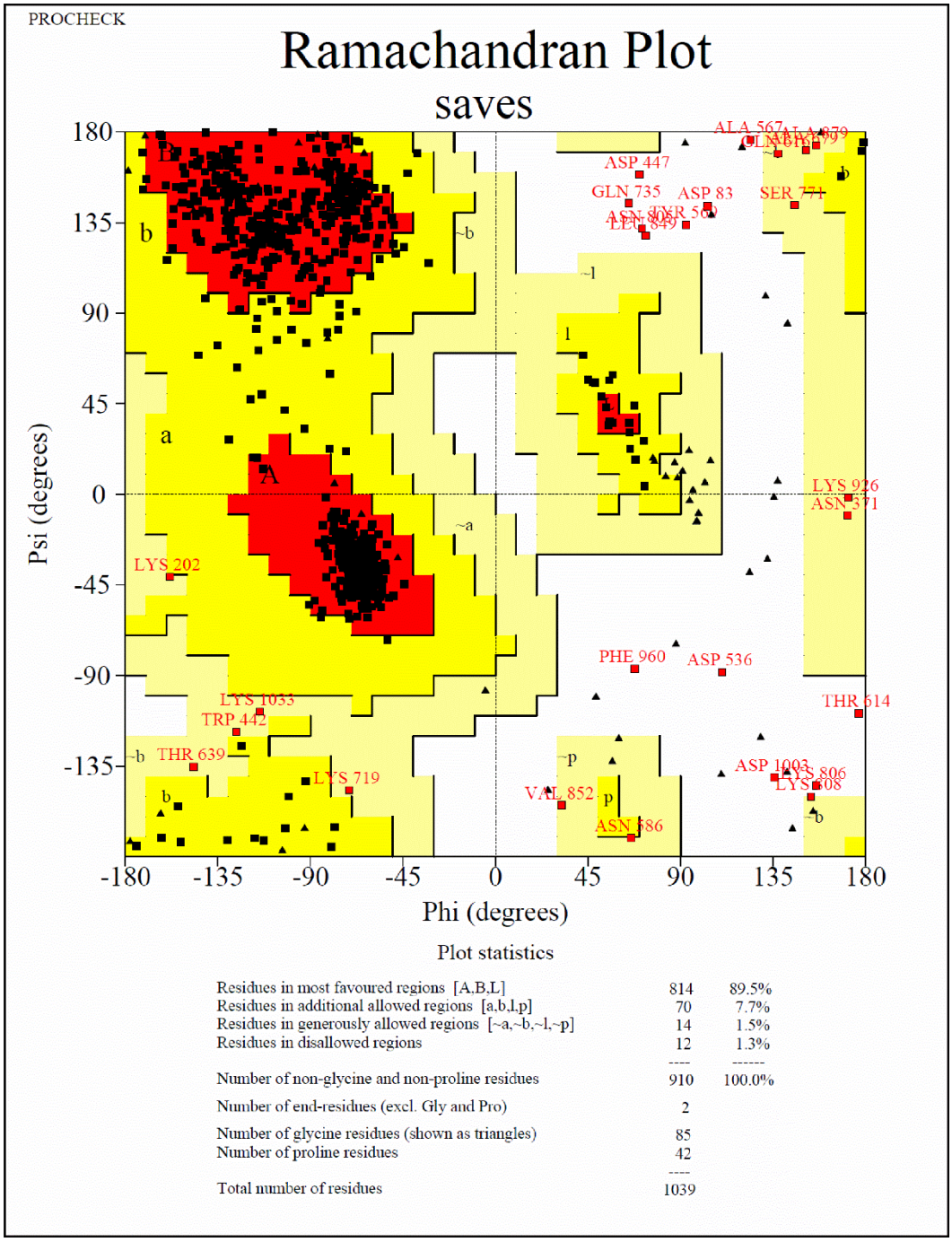

Figure S4. Graphical plot of the DLS analysis of the fusion protein. 10 cycles of 30s were used at a constant temperature of 25 °C.

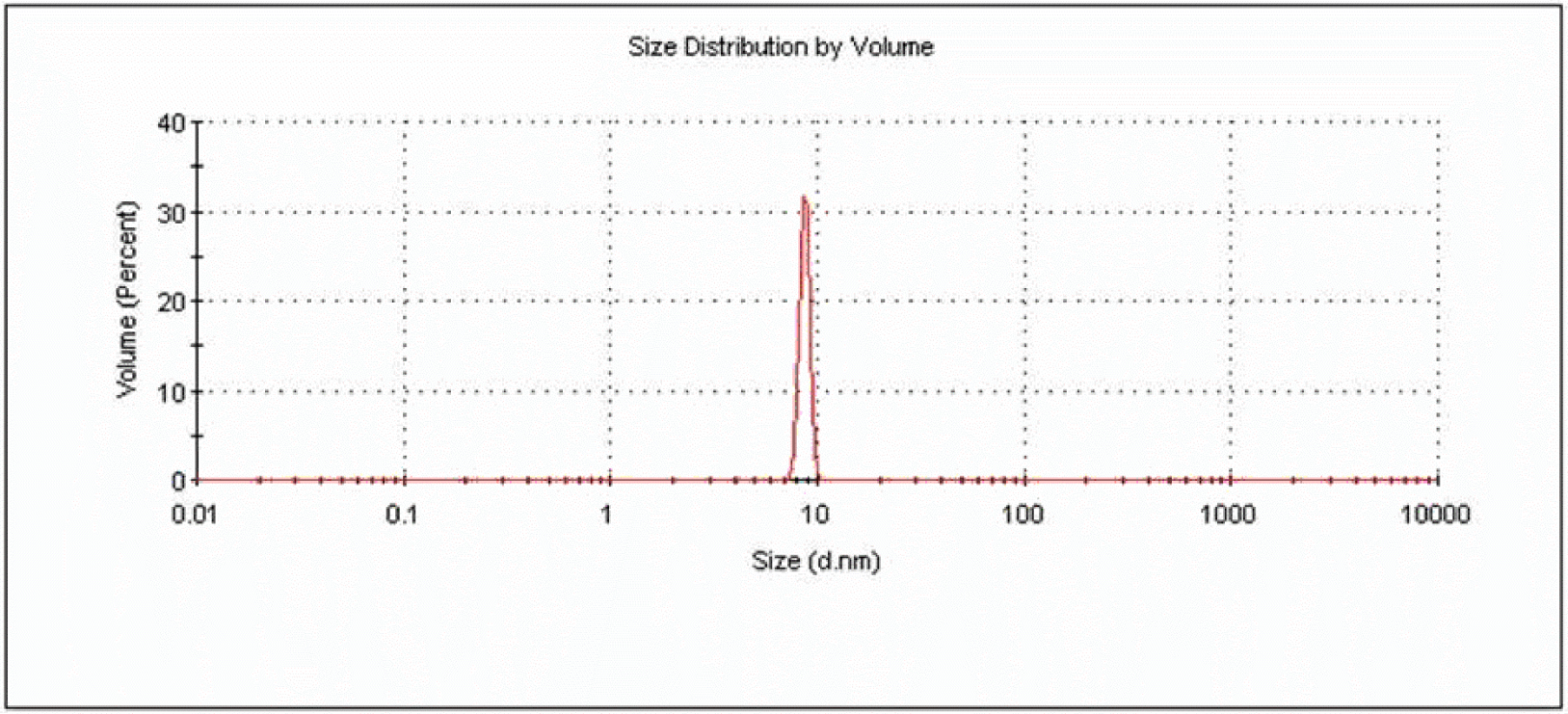

Figure S5. Characteristic CD-UV spectrum of the MBP:PLD:CP40 fusion protein, at 25°C in 20 Mm Tris and 100 mM NaCl (pH 7.4). The y-axis of the graph corresponds to the molar ellipticity at 103 deg.cm^2.dmol^{-1}, correlated with the progression of the wavelength, in nanometers.