Supplementary Material to the study entitled

„Structure and enzymatic mechanism of a moonlighting dUTPase"

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Supplementary Results: Mass spectrometry

Electrospray mass spectrometry has been shown to be capable of providing relevant information of oligomerization (protein complexation) characteristics while also having the great advantage of not depending on molecular shape and hydrodynamic properties of the macromolecules (Grandori *et al.*, 2009, Heck, 2008, Ngounou Wetie *et al.*, 2013, Benesch *et al.*, 2007). We wished to apply this technique to learn if it may be a convenient tool for characterizing trimeric dUTPases.

The electrospray mass spectrum of Φ11 phage dUTPase were measured on protein samples dissolved in volatile aqueous buffer (10 mM NH_4HCO3, pH 7.8) to approximate 'native' conditions while also allowing mass spectral measurements. Spectra presented in Fig. 2 clearly show the presence of an abundant species in the 3000-5000 m/z range. Charge distribution is narrow (mostly 16+, 17+ and 18+ charge states), which supports that the native protein conformation does survive transfer into the mass spectrometer. Mass difference between various charge states suggests that these are mostly ammonium ion adducts. Molecular mass of this species is 59,500 (±100) Da, corresponding to a non-covalent trimeric form of dUTPase, as based on the monomer molecular mass of 19,853, calculated from the amino acid sequence (cf Materials and Methods). Dimers, tetramers or other oligomers are not detected (their abundance is less, or much less than 5% that of the trimer). Absence of these oligomers confirms that the trimer is not an artifact (non-specific aggregate). In the low mass range (see insert in Fig. 2), the protonated monomer species is observed with a molecular mass 19,854 (±2) Da, in agreement with the amino acid sequence. Based on relative peak areas, monomer signals are only 5-10% that of the trimer, indicating high stability for the latter. In the low mass range signals corresponding to two impurities (molecular mass 19,154 and 17,425) are also observed (peaks indicated by "A" and "B" in Fig. 2). Trimers (or other oligomers) are observed only for the Φ11 phage dUTPase monomer, and not for these impurities. This, again, supports that Φ11 phage dUTPase is predominantly present in the solution as a trimer; and supports the conclusion that the observed trimer is not due to non-specific aggregation.

Supplementary References

Benesch, J. L. P., Ruotolo, B. T., Simmons, D. A. & Robinson, C. V. (2007). Chemical Reviews 107, 3544-3567.

Grandori, R., Santambrogio, C., Brocca, S., Invernizzi, G. & Lotti, M. (2009). Biotechnology Journal 4, 73-87.

Heck, A. J. R. (2008). Nature Methods 5, 927-933.

Ngounou Wetie, A. G., Sokolowska, I., Woods, A. G., Roy, U., Loo, J. A. & Darie, C. C. (2013). PROTEOMICS 13, 538-557.

Supplementary Table S1 List of primers used in site-directed mutagenesis experiments

Mutant	Primers
Φ11DUT^{F108W}	forward: 5′CTATATTACCCCGGGCGTGTGGGATATTAAAGGCGAAATTGATC3′
	reverse: 5′GATCAATTTCGCCTTTAATATCCCACACGCCCGGGGTAATATAG3′
Φ11DUT^{F164W}	forward: 5′GCGAACGCGGCGAAAAAGGCTGGGGCAGCAGCGGCGTG3′
	reverse: 5′CACGCCGCTGCTGCCCCAGCCTTTTTCGCCGCGTTCGC3′
Φ11DUT^{E158STOP}	forward: 5'GAATTTGAAAGCGTGAGCTAACGCGGCGAAAAAGGC3′
	reverse: 5′GCCTTTTTCGCCGCGTTAGCTCACGCTTTCAAATTC3'
Φ11DUT$^{\Delta 101-122}$	forward: 5'GCGATTGCGAGCAACTATGGCACCTATCAGATTAACGAAG3'
	reverse: 5'CTTCGTTAATCTGATAGGTGCCATAGTTGCTCGCAATCGC3'

Supplementary Table S2 Fluorescence spectral characteristics of dUTPases containing Trp residues in the apoenzyme and upon ligand binding

	hDUT^{F158W}	Φ11DUT^{WT}	Φ11DUT^{F108W}	Φ11DUT^{F164W}
F_{max}^{dUMP}	0.64[a]	1	0.96	0.897
F_{max}^{dUTP}	0.2[a]	0.97	1	0.67
F_{max}^{dUPNPP}	0.4[a]	0.94	0.94	0.64
λ_{max}^{apo} (nm)	353[a]	342	346	347
λ_{max}^{dUMP} (nm)	347[a]	342	346	347
λ_{max}^{dUTP} (nm)	339[a]	342	346	344
λ_{max}^{dUPNPP} (nm)	343[a]	343	346	342

[a] Published in (Tóth 2007 JBC)

F_{max} values indicate maximal fluorescence intensities observed upon saturation with the corresponding ligand, as compared to the fluorescence intensity of the apoenzyme, λ_{max} values indicate the wavelength of maximum fluorescence in the emission spectrum. Note fluorescence intensity changes and blue-shift of emission maximum in the Φ11DUT^{F164W} and the corresponding human dUTPase F158W construct, but not in the other Φ11 dUTPases.

Supplementary Figure Legends

Supplementary Figure S1 Mass spectrum of Φ11 phage dUTPase under native electrospray conditions.

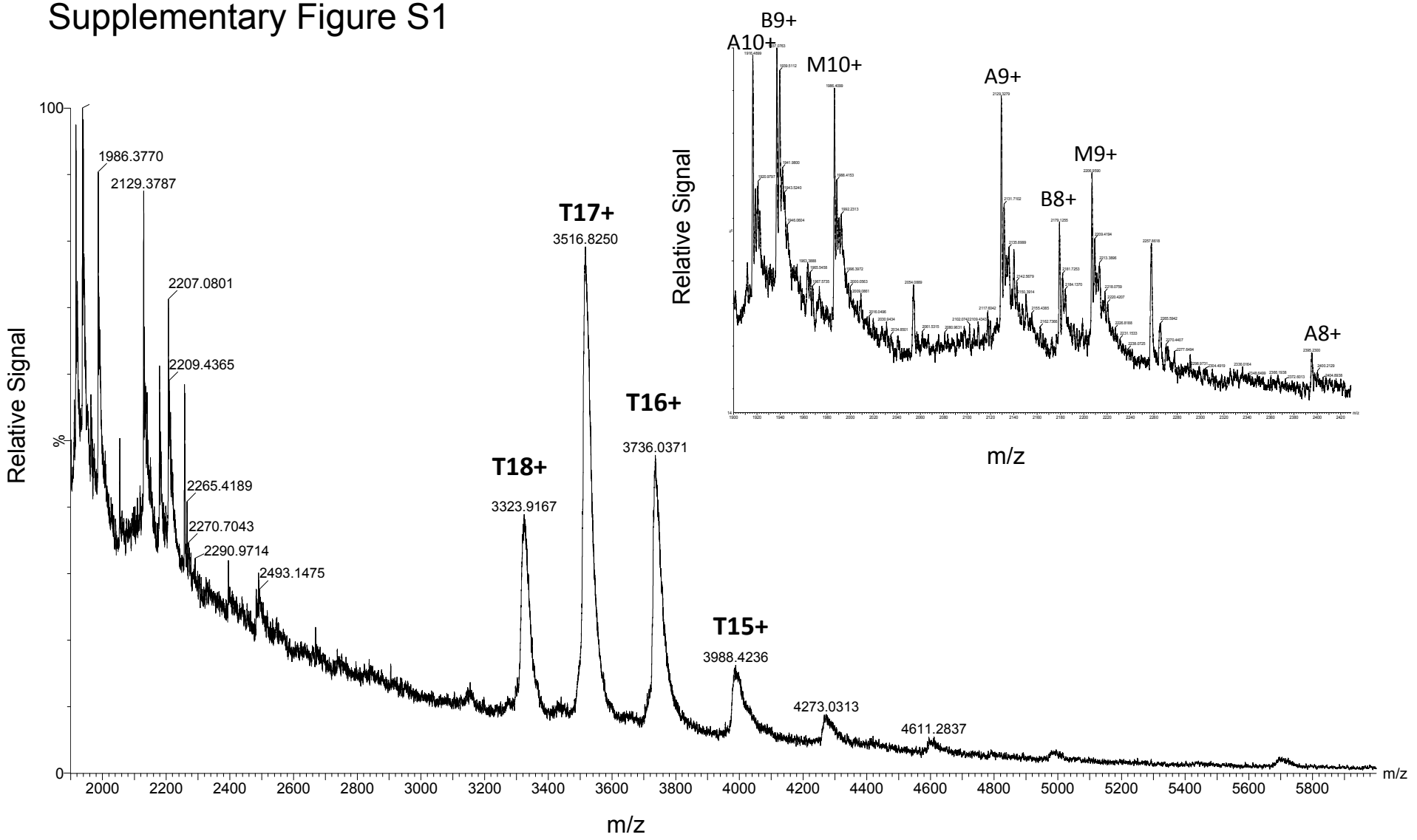
T17+, T16+ etc indicate trimer signals and 17+, 16+ etc charge states. Molecular mass of this species is determined to be 59,560 Da, corresponding to a trimer of the monomeric molecular mass of 19,854 Da. **Inset**: Monomer signals (M) and two small protein impurities (A and B) are also indicated; the numbers (e.g. A9+) indicate charge states. Molecular masses determined from the spectrum are 19,854, 19,154 and 17,425, for the dUTPase (M) and the two minor impurities (A and B), respectively.

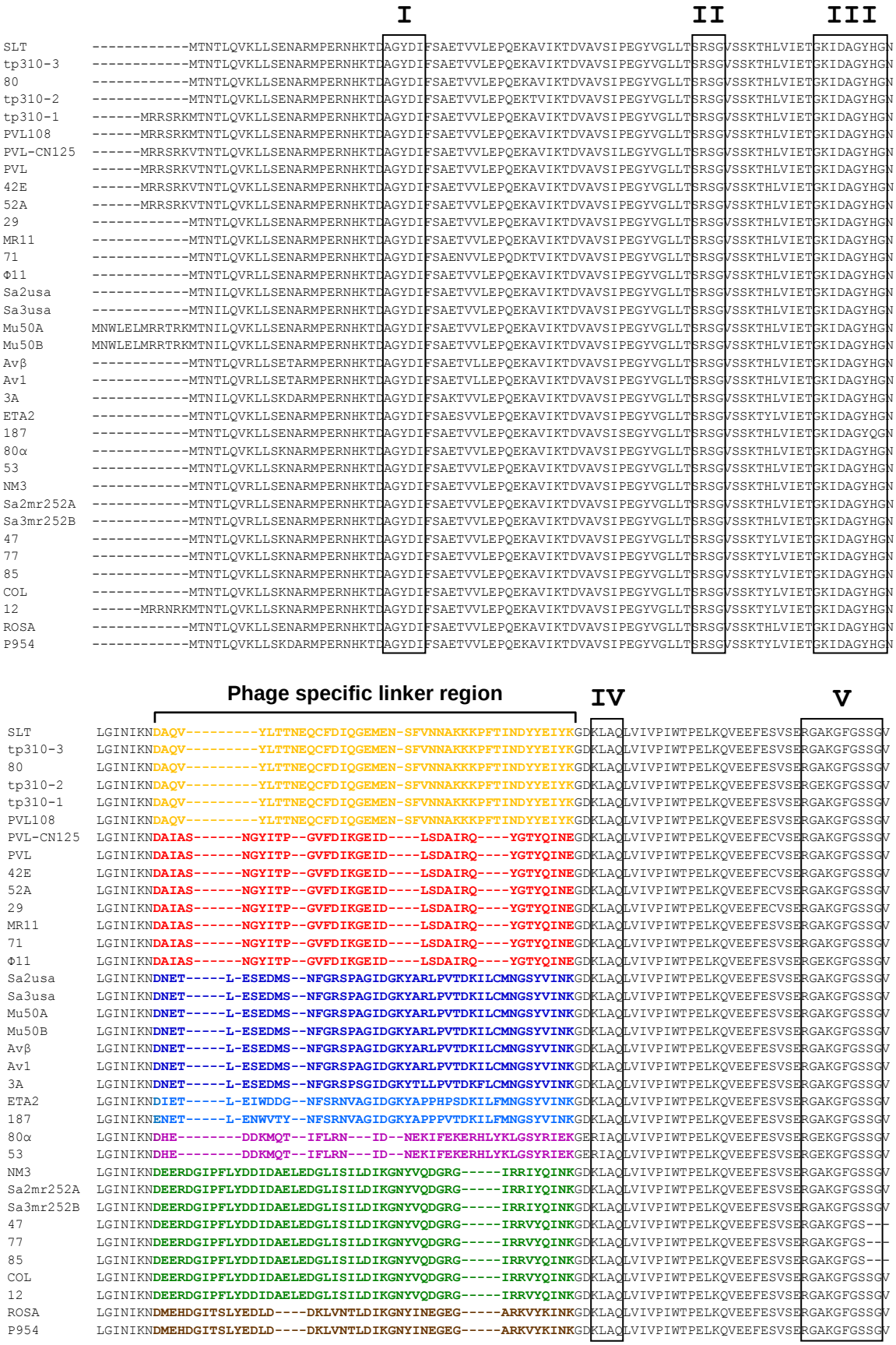
Supplementary Figure S2 Phylogenetic aligment of staphylococcal phage dUTPases

Supplementary Figure S3 Phylogenetic tree of staphylococcal phage dUTPases

Phage dUTPase sequences were analyzed by the ClustalX 2.1 with the neighbor-joining algorithm (cf Supplementary Fig. S2). Clusters of similar sequences are shown in different colours.

Supplementary Figure S1




I
II
III
Phage specific linker region
IV
V

Supplementary Figure S3