ESI: Supporting Information

for

Phosphorylated resveratrol as protein aggregation suppressor in vitro and in vivo

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Chemicals & Methods

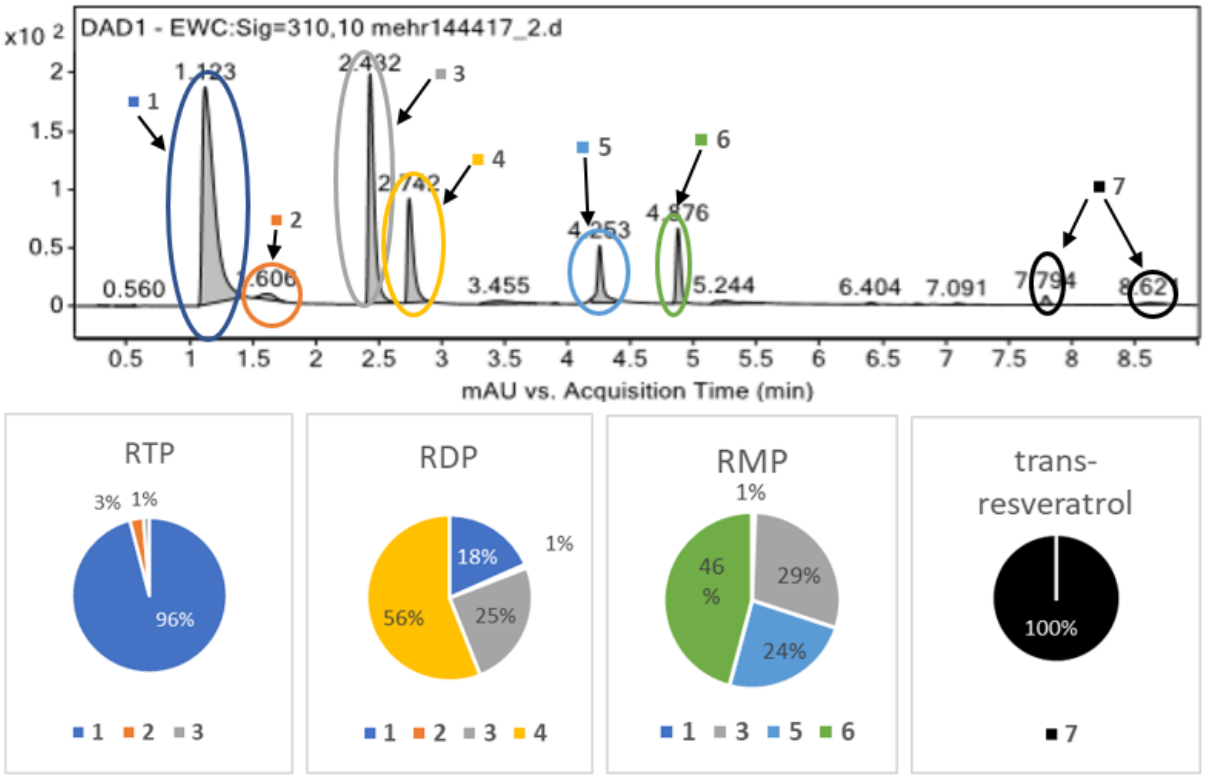
SI 1 Table giving the HPLC gradient for PR analysis

Time [min]	%A	%B
0	98	2
25	79	21
50	79	21
51	60	40
60	60	40
61	98	2
70	98	2

SI 2 Primer sequence for qPCR reaction in this work

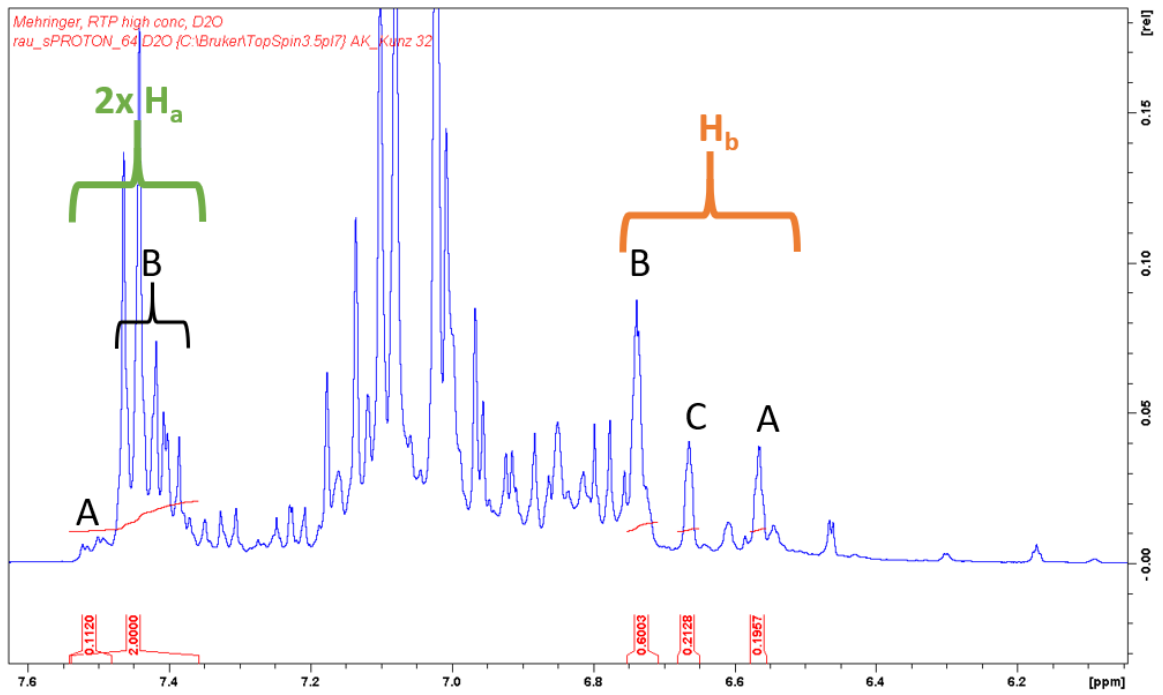
Primer	Sequence
ABpBACFW	ATGGCCCAGTTCCTGCGCC
ABpBACRV	TGCACCTCGTAACCGC
2xABFW	ACAAACCGCAAGAAAACAAC
2xABRV	AGGCATGATTTATGACTCTGAC
RP49RTFw	GCATACAGGCCCAAGATCCGT
RP49RTRv	CAATCTCCTTGCGCTTCTTG

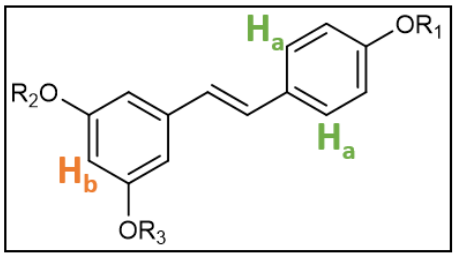
Additional information



SI 3 Chromatogram and LC-MS results for PR mixture.

Using liquid chromatography in combination with mass spectroscopy, the actual composition of the phosphorylated resveratrol (PR) mixture was analyzed. Expectedly, the mixture separated into rather distinctive peaks (see SI 3) as was reported in a patent on this compound (US 8,465,973 B3, Declerq et al.). However, the peak assignment in the patent appears to be rather crude, as our mass spectroscopy data revealed a poor separation of different PR derivatives leading to mixed peaks. This can be seen in SI 3, where the discrete mass signals for the derivatives of PR (namely RTP, RDP, RMP and trans-resveratrol) can be found not only in their designated main peaks but often also in preceding or trailing peaks. The compound RTP for example (identified by its distinctive mass) can be found mostly in the first peak, but also to a much lesser extent in the second and third peak. Similarly, RDP can be found mostly in two distinctive peaks (as was expected for the two structural isomers of RDP, cf. Figure 1a), but also to a significant amount in the first RTP main peak. Pie-charts accompanying the chromatograms in SI 3 give an impression of the distribution of each derivative across the peaks as determined by HPLC area. Taking in to account the varying extinction coefficients for different PR derivatives[1], no quantitative results can be gathered from LC-MS measurements. However, it shows that different PR species appear to have a strong interaction with each other, making a separation in HPLC difficult and incomplete. This suggests, that PR compounds can also have strong interactions with various other compounds that share similar features (e.g. extended π-systems), which is important for potential mechanisms in in-vitro and in-vivo applications.





	H	Phosphate	H/Phosphate
NMR Peaks	A	B	C
R_1	5.6%	94.6%	
R_2 & R_3	19.3%	59.3%	21.3%

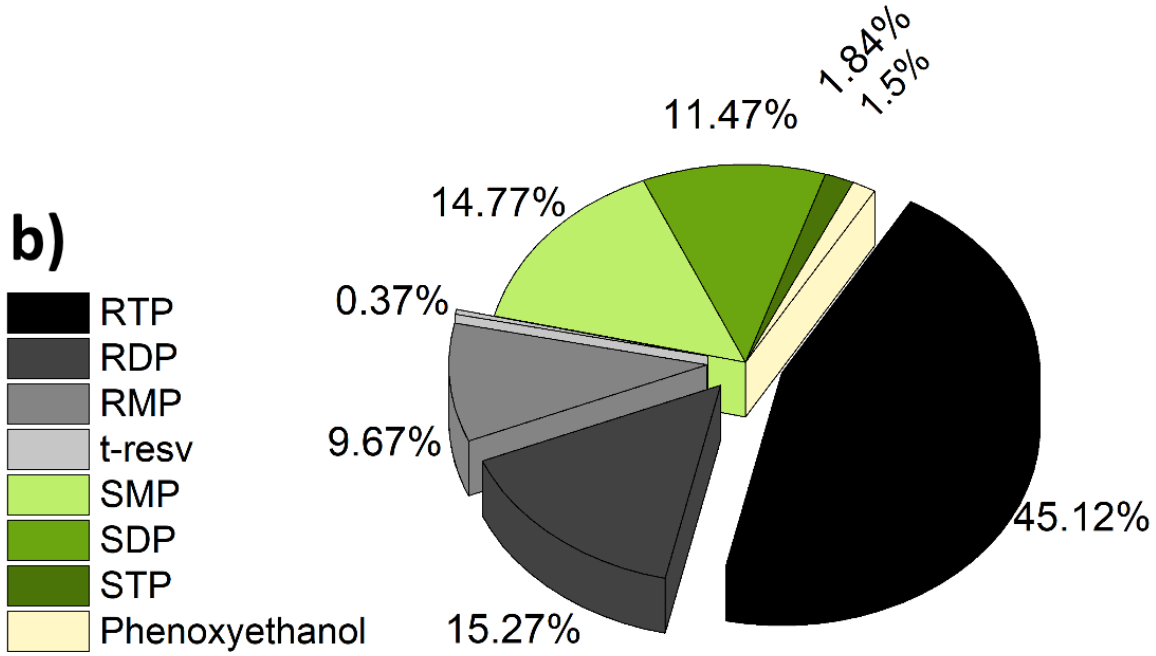
SI 4 Proton NMR analysis of PR mixture. Relative integrals of distinctive protons yield the ratios of phosphorylated/unphosphorylated ester bonds.

In order to determine the composition of the PR mixture as well as identifying impurities, NMR measurements were conducted. The proton NMR was found to be relatively messy at first glance, but this can be explained by considering the mixture of PR derivatives present in the sample. The number of different permutations (number and position of phosphate groups) inevitably lead to an overlay of NMR signals from various compounds that are shifted slightly up or downfield. This makes correct peaks assignment almost impossible for most protons. However, two proton types (H_a and H_b) are conveniently located at either end of the spectrum and relate to positions in either ring (see SI 4). Using the chemical shifts reported for pure compounds by Aleo et al.[1], the peaks and their integrals could be assigned to different PR subspecies, which allowed the calculation of phosphorylated/unphosphorylated hydroxy group ratios as shown in SI 4. Using an internal standard, the molar purity in regards to resveratrol-bearing structures was calculated to be about 60% of what would have been expected for a pure compound containing RTP exclusively.

a)

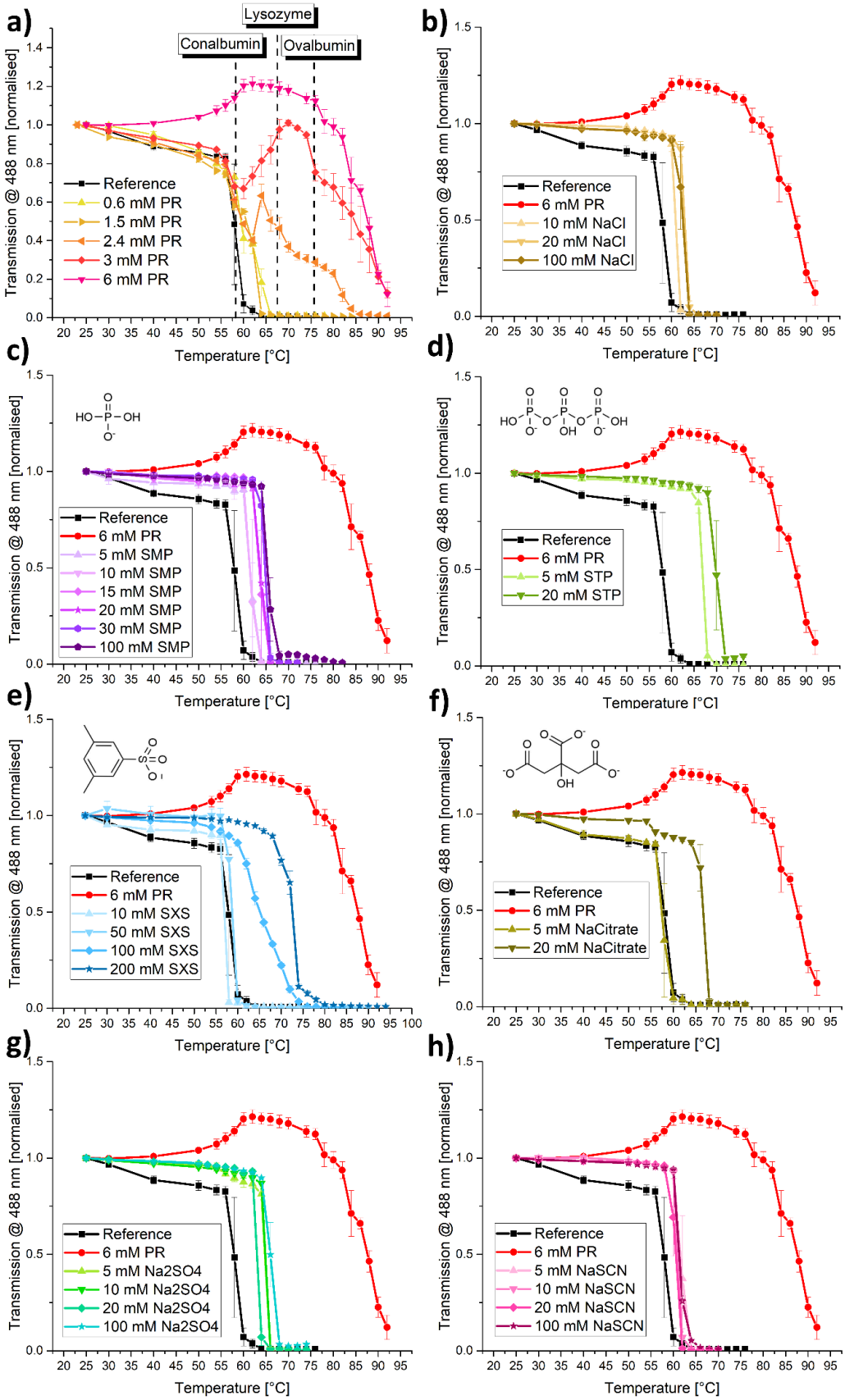
Compound	Content
t-resveratrol	1.1%
RMP	19.4%
RDP	23.5%
RTP	56.1%
Total phosphorylation degree (calculated)	78.2%
Measured phosphorylation (^{31}P-NMR)	80.3%

b)

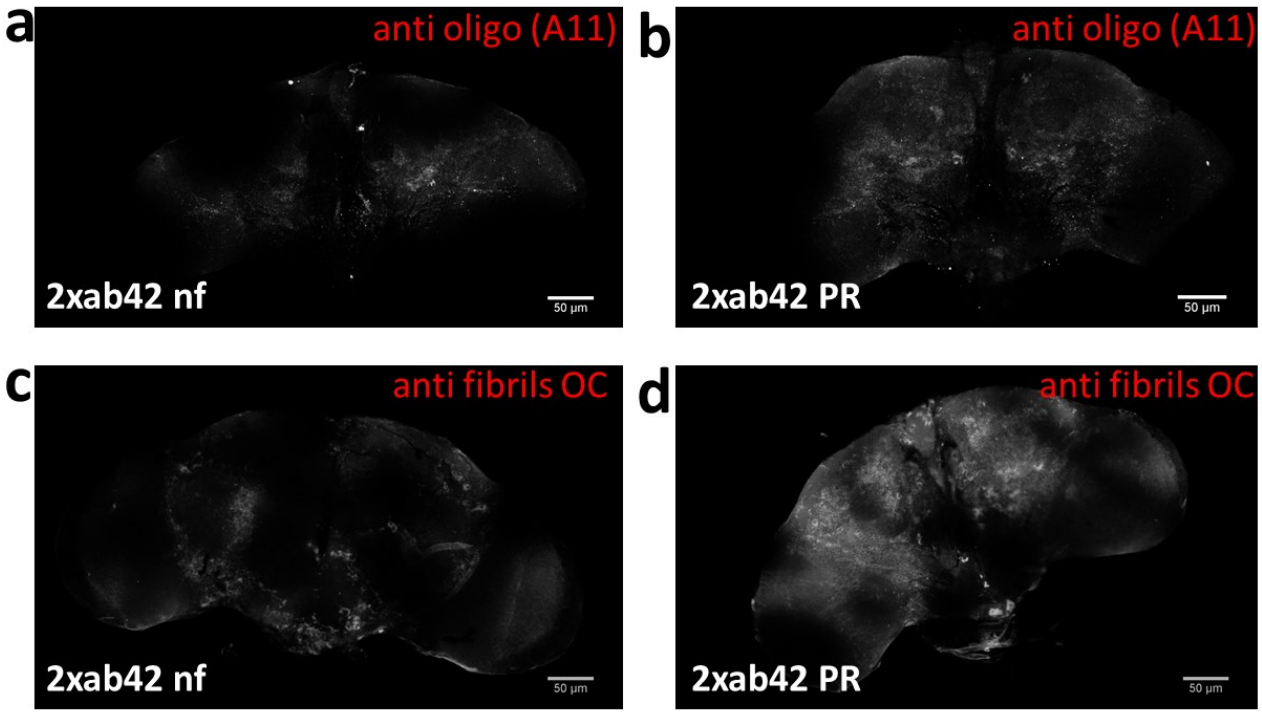


SI 5 Composition of PR mixture. ***a:*** *calculated composition of resveratrol-bearing derivatives based on the phosphorylated/unphosphorylated ester bond ratios in SI 4. Below is the theoretical total degree of phosphorylation based on the calculated composition with the actual measured phosphorylation degree as per ^{31}P-NMR.* ***b:*** *Total composition of PR mixture including inorganic impurities and traces of phenoxyethanol in mass percent.*

Using the ratios of phosphorylated ester bonds in SI 4 as a basis, the percentages of t-resveratrol, RMP, RDP and RTP could be calculated and are given in SI 5 a. Using these figures, the degree of total phosphorylation in regards to the resveratrol-bearing structures present can be calculated and amounts to approximately 78%. This is in good agreement with ^{31}P-NMR measurements that gave a phosphorylation degree of about 80 % (using an internal standard). This translates to an average of 2.4 phosphate units per resveratrol structure. As further impurities, proton NMR revealed traces of phenoxyethanol (as per manufacturer data sheet) and ^{31}P-NMR showed significant amounts of inorganic mono-, di- and traces of tri- phosphate. The total makeup of the compound can be found in SI 5 b in mass percent. This concludes to about 70 wt% purity in regards to PR which matches the manufacturers specification (60-85%).



SI 6 Turbidity of CCEW solutions (pH 7.4) with various ionic additives in dependence of temperature. Error bars mean ± SD (N=3). For some non-self-explanatory compounds, the molecular structures in ionic form are displayed as insets and the 6 mM PR curve is replicated in each graph to aid the reader. ***a:*** *PR (copy of Figure 3 a for convenience),* ***b:*** *sodium chloride,* ***c:*** *sodium mono phosphate,* ***d:*** *sodium tri phosphate,* ***e:*** *sodium xylene sulfonate,* ***f:*** *sodium citrate,* ***g:*** *sodium sulfate and* ***h:*** *sodium thiocyanate.*



SI 7 Confocal microscopy pictures (max. intensity projection) of drosophila brains exemplifying the difference in sequence specific antibody binding between the two food groups. ***a & b:*** *anti-oligomer immunostained brains of nf (**a**) or PR (**b**) supplemented flies.* ***c & d:*** *anti-fiber OC immunostained brains of nf (**c**) or PR (**d**) supplemented flies.*

References

1. Aleo D, Cardile V, Chillemi R, Granata G, Sciuto S. Chemoenzymatic Synthesis and Some Biological Properties of O-phosphoryl Derivatives of (E)-resveratrol. Nat Prod Commun. 2008;3(10):1693–700.

2. Jameson LP, Smith NW, Dzyuba S V. Dye-binding assays for evaluation of the effects of small molecule inhibitors on amyloid (Aβ) self-assembly. ACS Chem Neurosci. 2012;3(11):807–19.