Electronic Supplementary Material (ESI) for Analytical Methods.
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Supplementary Information

Analytical Methods

Evaluation of different strategies for Selenomethionine (SeMet) analyses in selenized yeast by asymmetrical flow field flow fractionation coupled to inductively coupled plasma mass spectrometry (AF4-ICP-MS).

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Table S1 SeMet molecular size from different sample preparation in 10 kDa RC membrane estimated by Berry model first-order fit using MALS detector and Astra 5 software.

Sample preparation	RMS/nm ± RSD
FA, not filtered	163 ± 5
FA, filtered	160 ± 5
MSA, no refluxed and no filtered	155 ± 4
MSA, no refluxed and filtered	209 ± 16
MSA, refluxed and no filtered	140 ± 9
MSA, refluxed and filtered	138 ± 16
SDS buffer, not filtered	482 ± 11
SDS buffer, filtered	356 ± 6
Water, not filtered	164 ± 7
Water, filtered	ND
Water, with ultrasonic bath and not filtered	146 ± 4
Water, with ultrasonic bath and filtered	ND
Water, with ultrasonic probe and not filtered	149 ± 11
Water, with ultrasonic probe and filtered	ND

ND: not detected by MALS; RMS: root-mean-square radius; FA: formic acid; MSA: methane sulfonic acid; SDS: sodium dodecyl sulfate; RSD: relative standard deviation.

Table S2 Comparison of different methodologies for SeMet analyses in different yeast samples.

Sample	Form of Se as presented on label	Labeled Se[a] (µg Se/tablet)	Average tablet mass (g)	SeMet (µg Se/tablet) ± SD	
				FFF-ICP-MS	HPLC-ICP-MS[b]
1	Selenium (yeast)	200	0.346	147 ± 30	135.1 ± 26.9
2	SelenoExcell® selenium (as high selenium yeast)	200	0.504	142.5 ± 24	131.2 ± 20.2

[a] total selenium is the sum of Se methionine and inorganic selenium
[b] data from LeBlanc et al [43] (sample 1 refers to S3, sample 2 refers to S6)
SD: standard deviation

Fig. S1 Fractionation analysis where were collected three fraction of the cross flow waste (during the injection, focus and elution steps) and one fraction of the detector waste (corresponding to the elution peak)

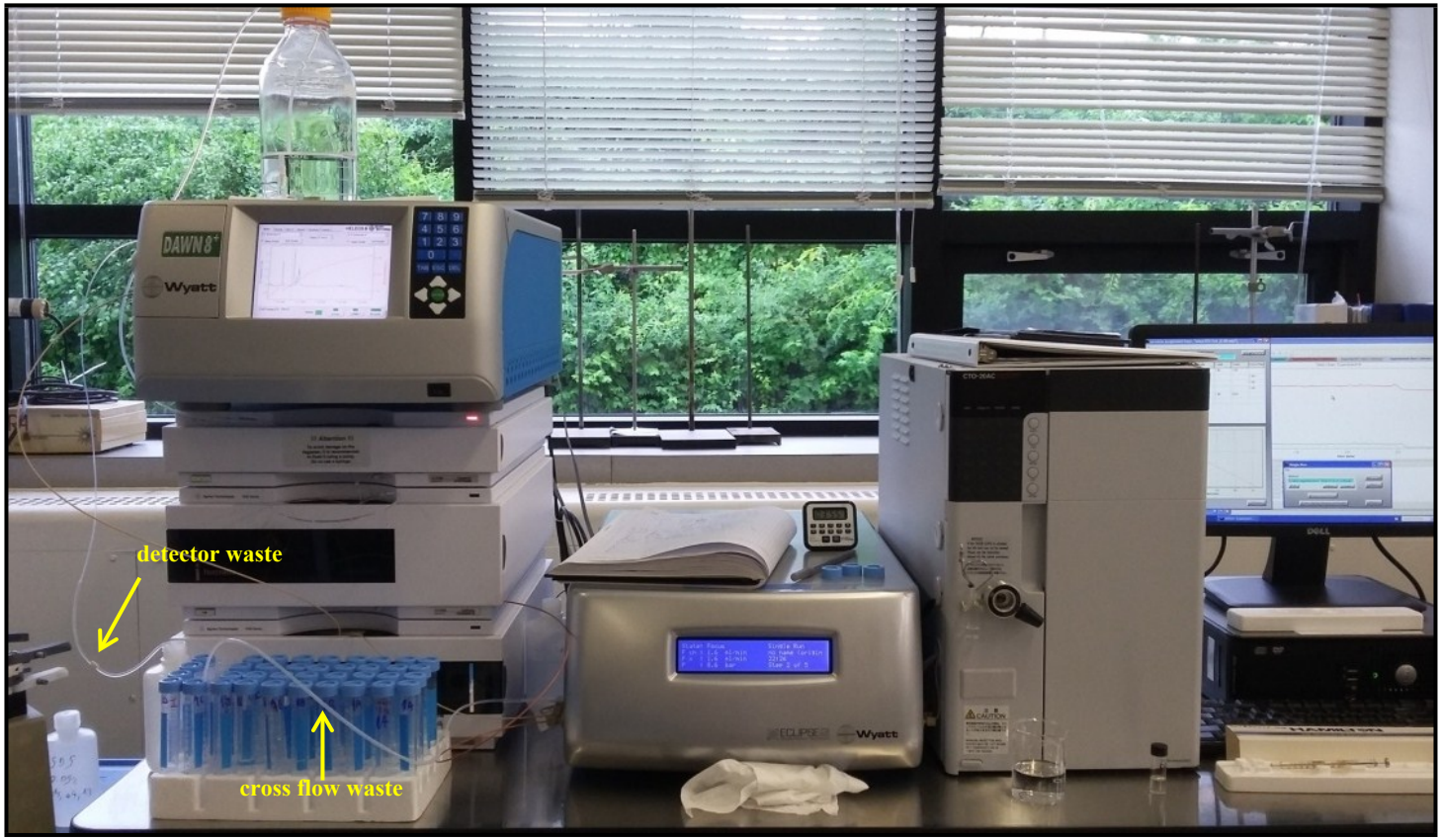


Fig. S2 SeMet containing molecule fractograms for different sample preparation obtained by MALS detector and 10 kDa RC membrane. Extraction in (a) formic acid; (b) methanesulfonic acid without reflux; (c) methanesulfonic acid with reflux;(d) sodium dodecyl sulfate buffer; (e) Water

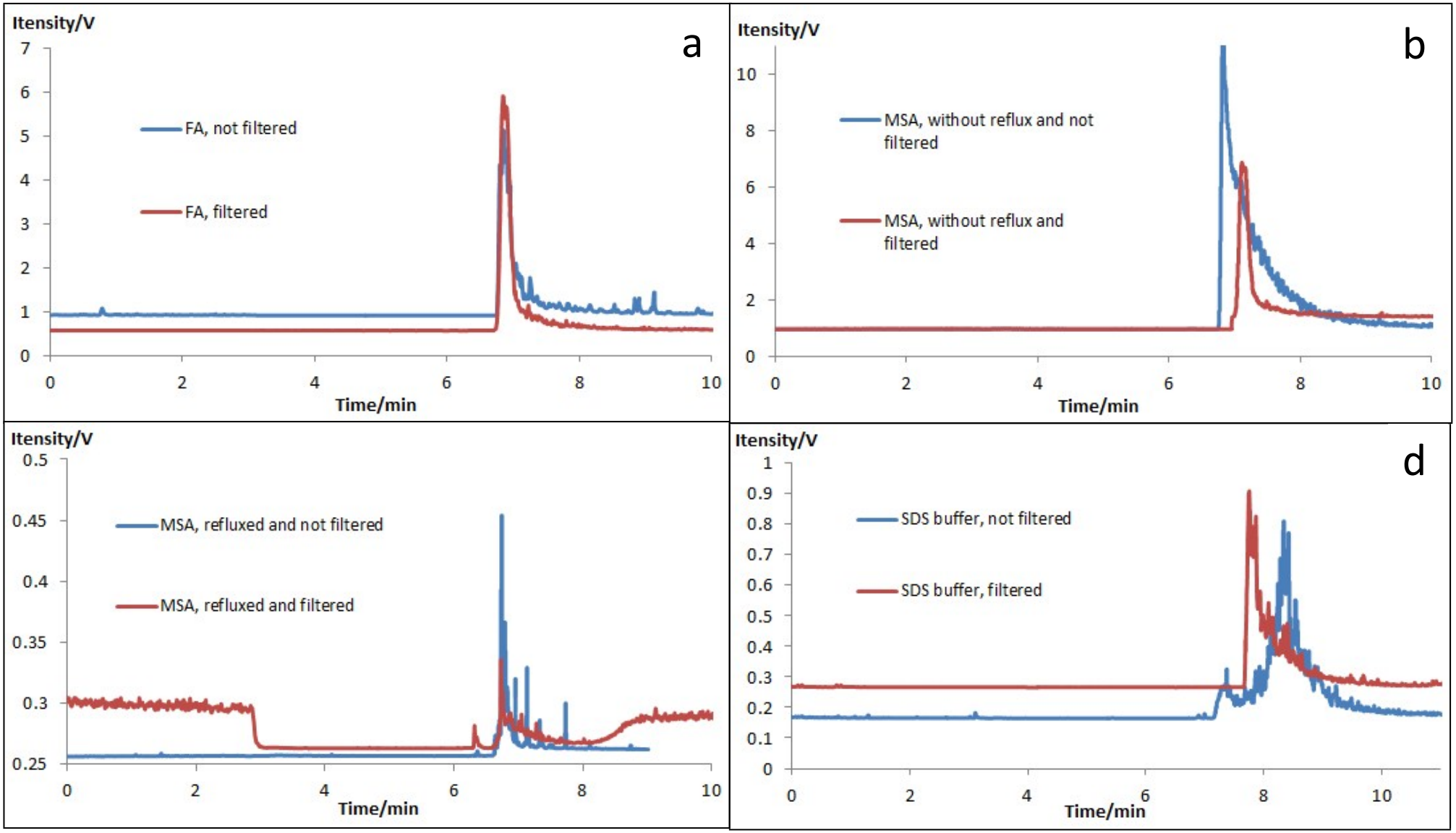
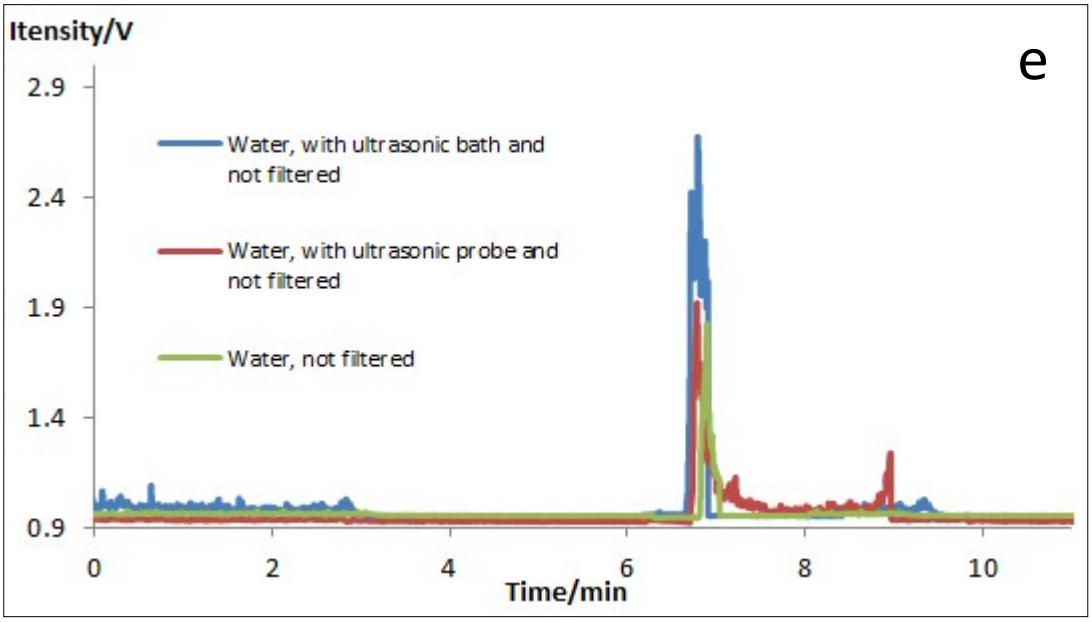


Fig. S2 (*continued*)



Note: The digestion using SDS buffer was diluted 50 times before injection;

MALS: Multi-Angle Light Scattering; FA: formic acid; MSA: methane sulfonic acid; SDS: sodium dodecyl sulfate;

Retention time/min: FA not filtered: 6.851;

FA filtered: 6.834;

MSA not filtered and not refluxed: 6.810;

MSA filtered and not refluxed: 7.096;

MSA not filtered and refluxed: 6.736;

MSA filtered and refluxed: 6.728;

SDS buffer not filtered: 8.325;

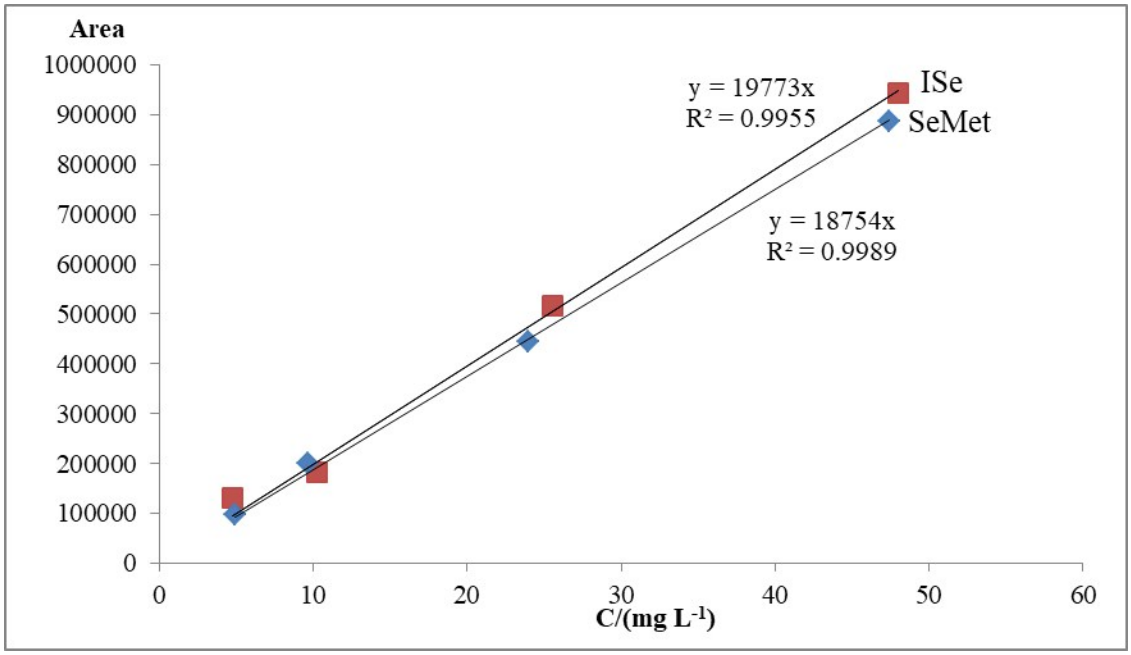
SDS filtered: 7.743;

Water with ultrasonic bath and not filtered: 6.785;

Water with ultrasonic probe and not filtered: 6.777;

Water: 6.892.

Fig. S3 Comparison of angular coefficients of two Se species. Curves obtained by FIAS system



Note: Se: selenium; ISe: inorganic selenium; SeMet: selenomethionine; FIAS: flow injection analysis system.

- Statistical 'Hartley F test' (for 2 freedom degrees and 95% confidence):

$$F_{calc} = {}^{S^2_{higher\ ang.\ coef.}}\Big/_{S^2_{lower\ ang.\ coef.}} = {}^{19773^2}\Big/_{18754^2} = 1.11; \quad F_{critical} = 19$$

As $F_{calc} < F_{critical}$ there is no significant difference, which means, whatever the curve used to quantify SeMet, the results will be similar within the statistical error.