SUPPLEMENTARY MATERIAL

Application of the SMALP technology to the isolation of GPCRs from low-yielding cell lines

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S1 Synthesis and characterization of SMAc co-polymer C30

The synthesis of N30 by co-polymerization of Sty with MAn (Sty/MAn ratio: 8:1, n/n) was carried out in THF solution under radical conditions in the presence of AIBN (4%, n/n) as thermal initiator, achieving a yield of 35%. The occurrence of polymerization involving the double bonds was confirmed by ^{1}H-NMR analysis, which showed the lack of the resonances of vinylic protons of MAn and Sty (around 7.1, 5.7 and 5.2 ppm, respectively). Furthermore, a new signal appeared in the 1–2 ppm region belonging to the aliphatic main chain (Figure S1). The final molar composition of N30 was assessed by ^{13}C-NMR by comparing the integrated peak areas of MAn carbonyl carbon atoms, located at around 173 ppm, to those related to the aromatic carbon atoms of Sty co-units in the range 150–120 ppm (Figures S2 and S3). The pre-hydrolyzed copolymer showed a MAn content of 30% (Sty/MAn ratio: 2.3:1, n/n).

N30 was then hydrolyzed to C30 in alkaline solution [1]; the reaction was boosted by subsequent additions of NaOH and by increasing the reaction time (5 days). The ATR-FTIR analysis of the obtained white solid (Figure S7) confirmed the presence of the SMAc co-polymer: the absence of the signals at 1855 and 1775 cm^{-1} related to the C=O stretching of MAn (Figure S4), and the presence of the signal at 1565 cm^{-1} related to the COO^{-} stretching of maleic acid (MAc) reasonably led us to assume that the hydrolytic cleavage was taken to completion. On the other hand, the ^{13}C-NMR spectrum showed a downfield shift of the resonances of the carbonyl carbon atoms (Figure S6). The integration of the ^{13}C-NMR spectrum confirmed that the Sty/MAc ratio was maintained after hydrolysis. The average molecular weights of C30 were assessed by SEC analysis in THF solution at 25 °C ($\bar{M}_n$ = 6000 g/mol; $\bar{M}_w$ = 11500 g/mol).

SMAn co-polymer N30. *^{1}H-NMR (acetone-d_6):* 7.8–5.6 (arom. St, CH), 4.2–0.6 (main chain, CH, CH_2) ppm. *^{13}C-NMR (acetone-d_6):* 172.5 (MA, C=O), 147.3–133.8 (arom. St, Cq), 131.1–124.7 (arom. St, CH), 54.6–31.6 (main chain CH, CH_2), ppm.

SMAc co-polymer C30. Details reported in the main text (section 2.3).

References

[1] S.C. Lee, T.J. Knowles, V.L.G. Postis, M. Jamshad, R.A. Parslow, Y. Lin, A. Goldman, P. Sridhar, M. Overduin, S.P. Muench, T.R. Dafforn, A method for detergent-free isolation of membrane proteins in their local lipid environment, Nat. Protoc. 11 (2016) 1149–1162. https://doi.org/10.1038/nprot.2016.070.

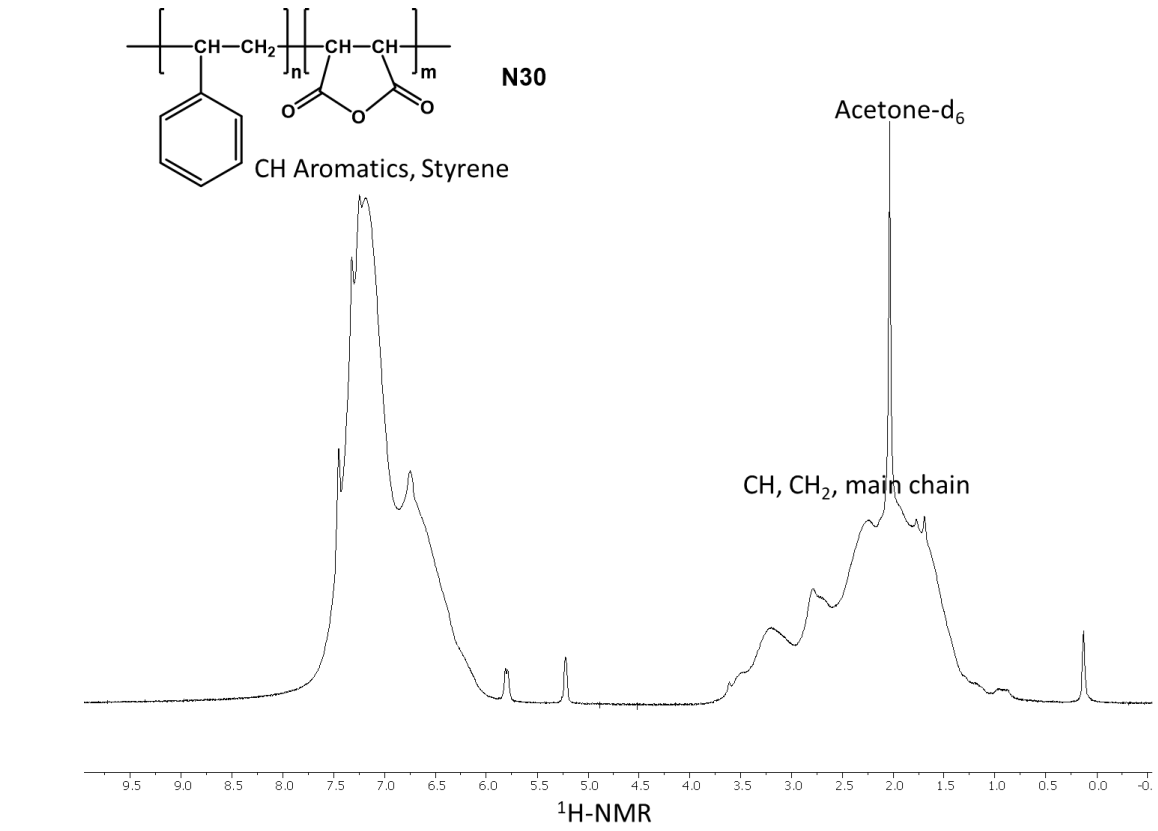

Figure S1. ^{1}H-NMR spectrum of SMAn co-polymer **N30** [solvent: Cr(acac)$_3$ 0.1 M in acetone-d$_6$].

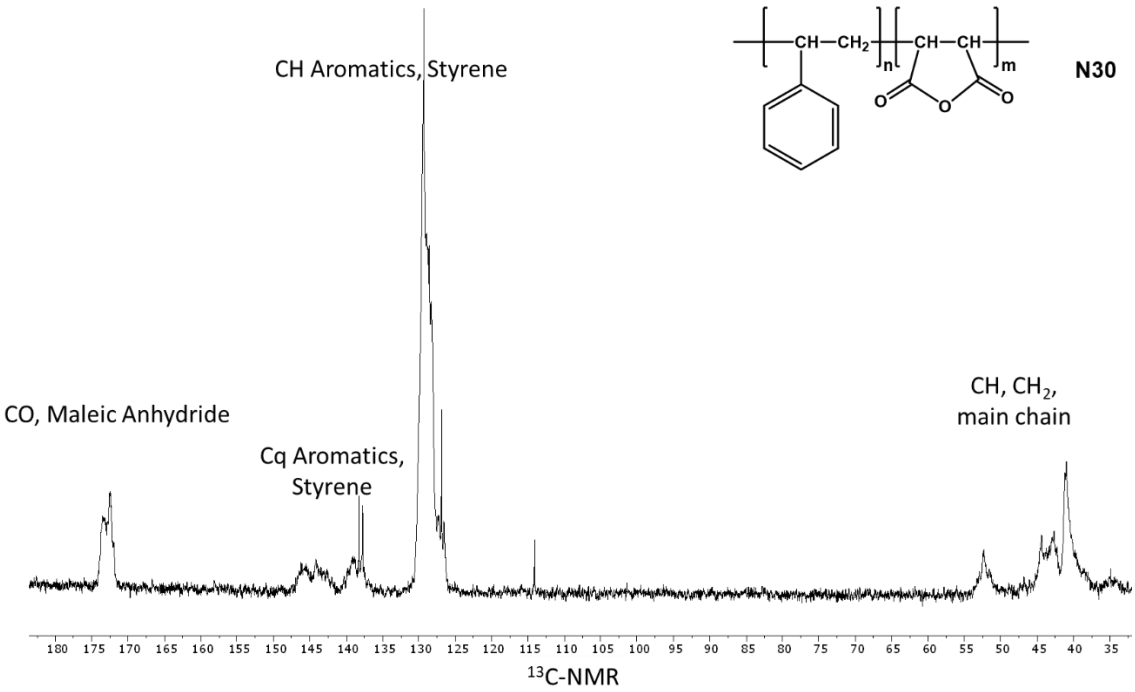

Figure S2. ^{13}C-NMR spectrum of SMAn co-polymer **N30** [solvent: Cr(acac)$_3$ 0.1 M in acetone-d$_6$].

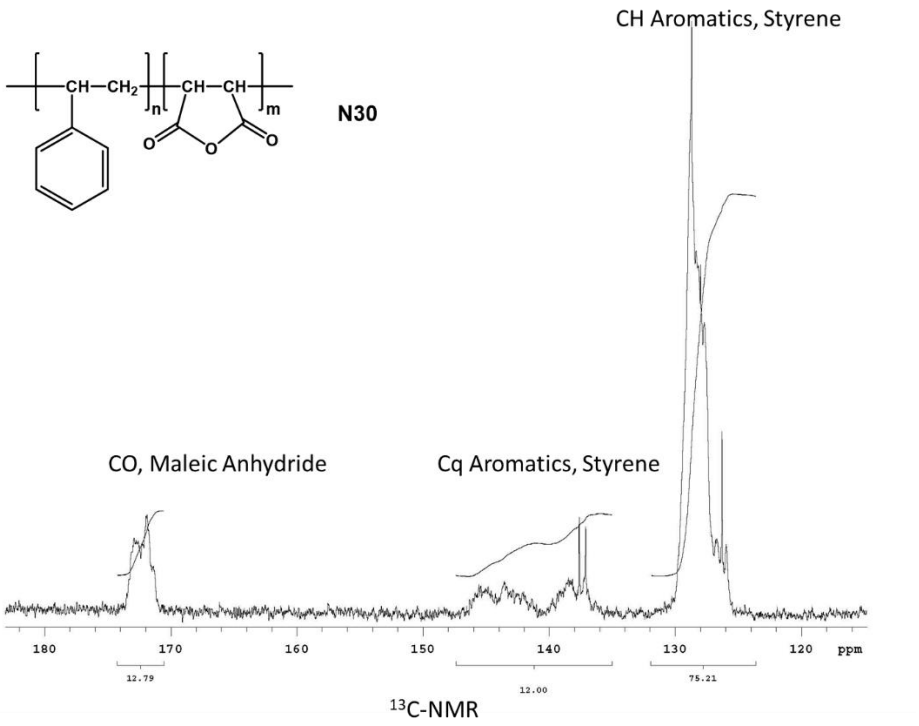

Figure S3. ^{13}C-NMR spectrum of SMAn co-polymer **N30** [solvent: Cr(acac)$_3$ 0.1 M in acetone-d$_6$] enlargment and integrations.

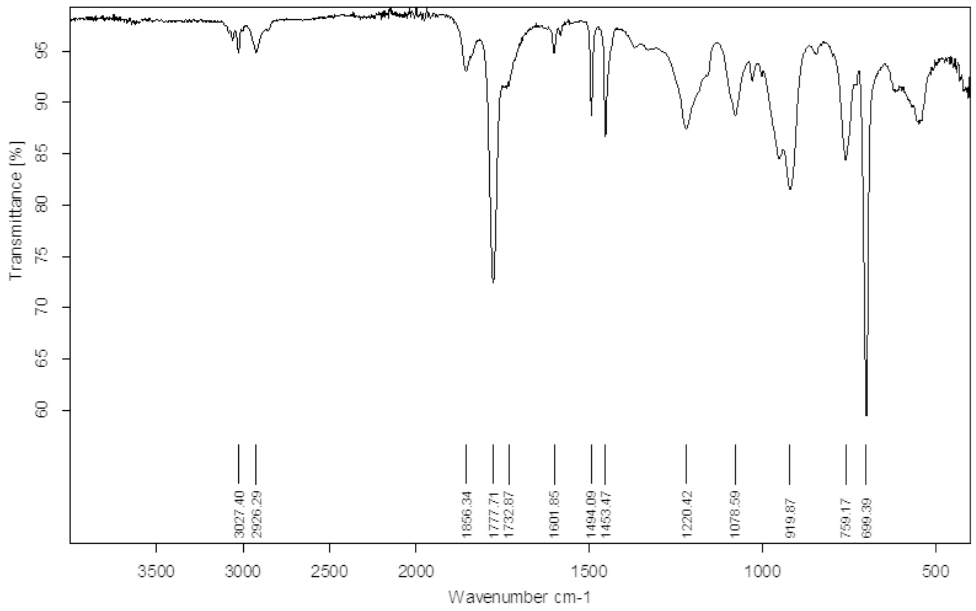

Figure S4. ATR-FTIR spectrum of SMAn co-polymer **N30**.

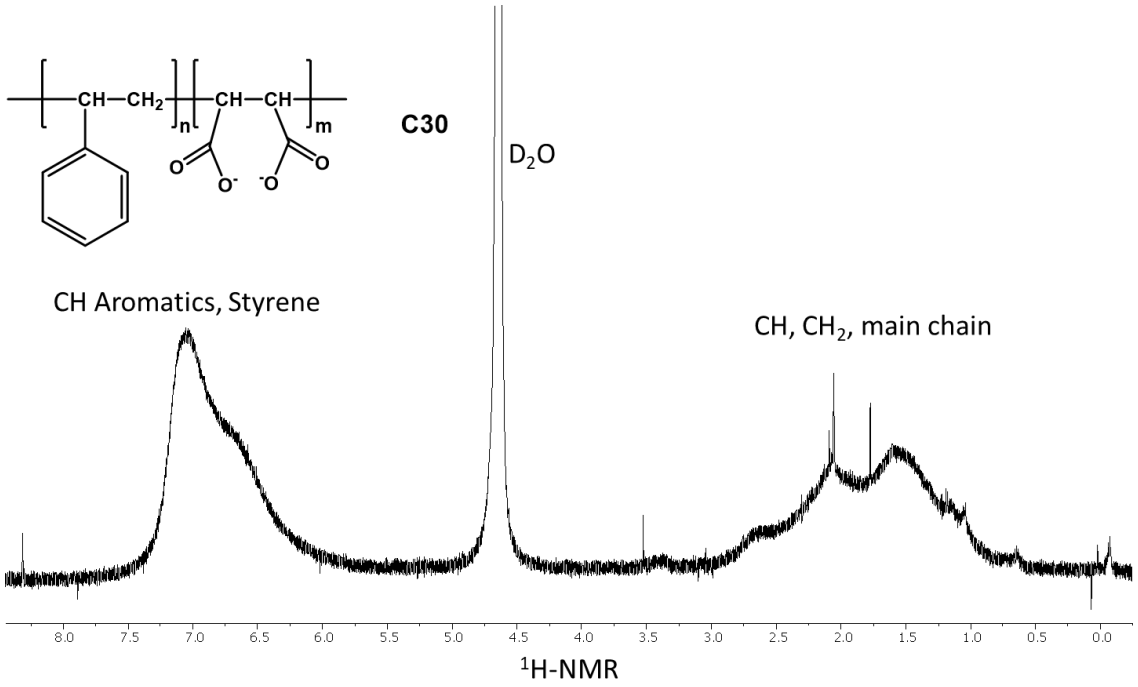

Figure S5. ^{1}H-NMR spectrum of SMAc co-polymer **C30** [solvent: D_2O].

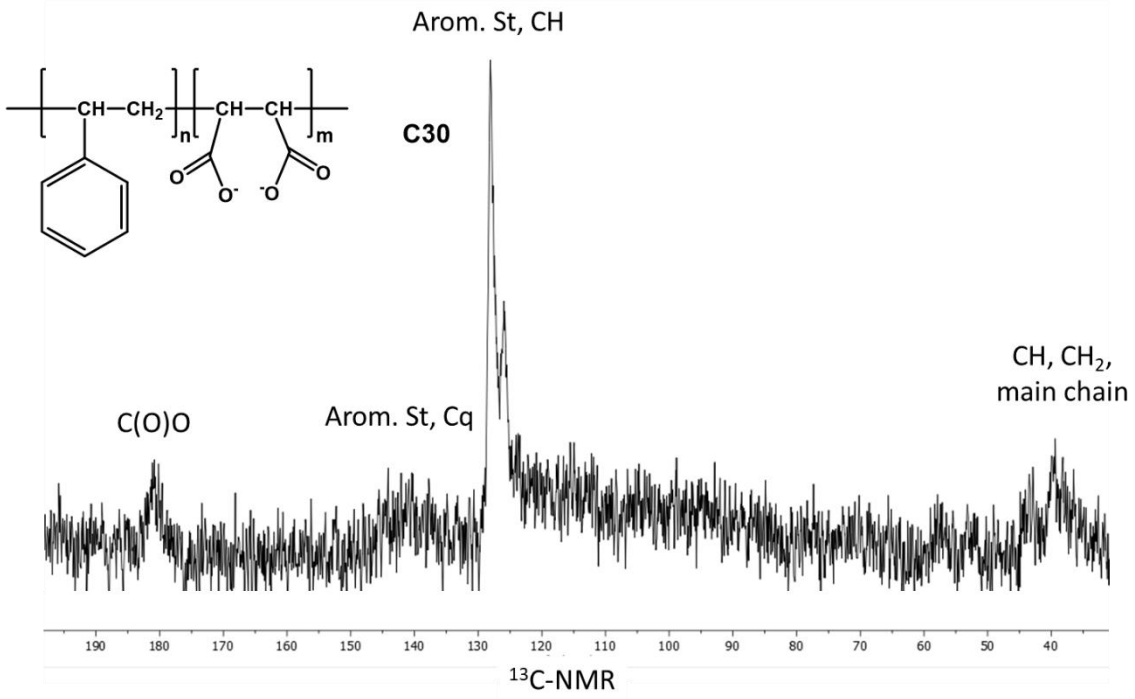

Figure S6. ^{13}C-NMR spectrum of SMAc co-polymer **C30** [solvent: D_2O].

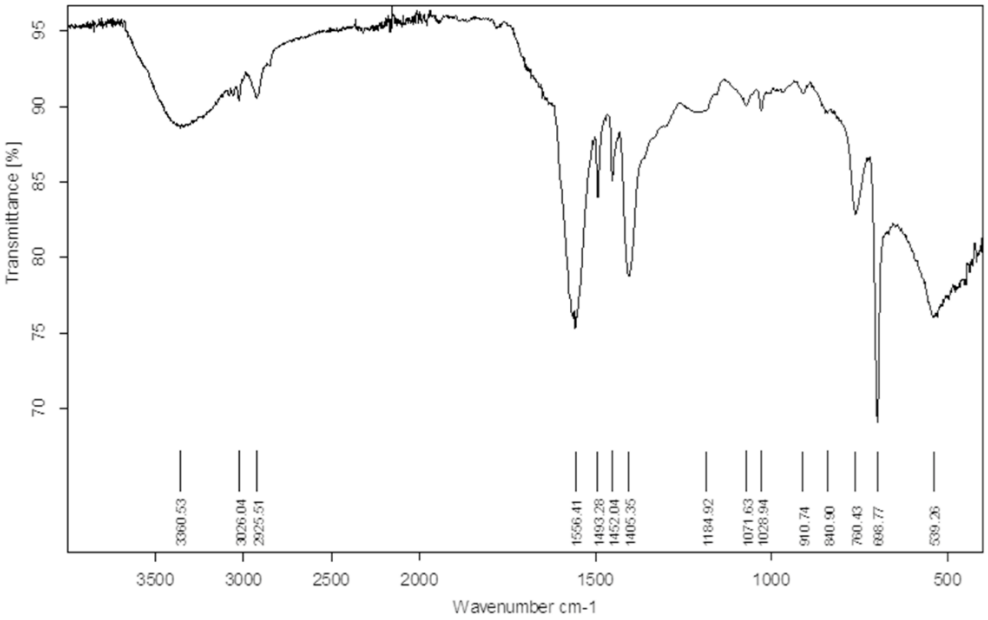

Figure S7. ATR-FTIR spectrum of SMAc co-polymer **C30**.

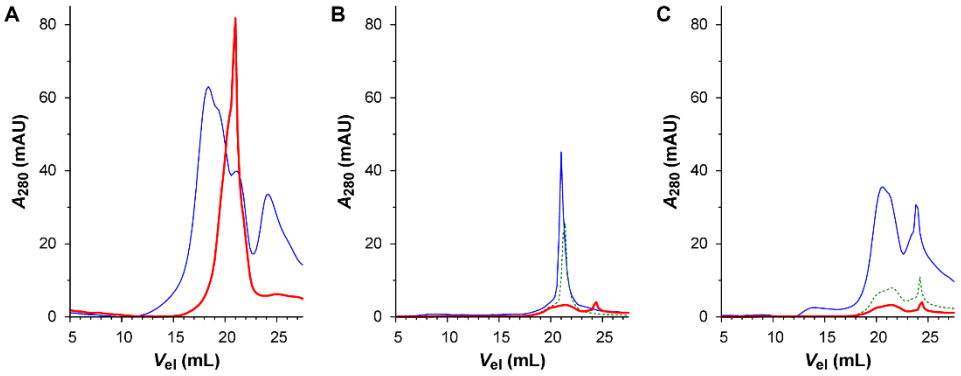

Figure S8. Chromatograms (280 nm) of SMAc co-polymer X30 in KP10 buffer (pH 8.0) mixed 1:1 (v/v) with SMALP buffers, as obtained by SEC analysis on a Superdex 200 10/300 GL column. (**A**) Samples were prepared with X30 4% (m/v) and SB500 buffer; SEC runs were performed using RB500 (*thin blue*) and RB0 (*bold red*) buffers as mobile phases. (**B**) Samples were prepared with X30 0.8% (m/v) and SB500 (*thin blue*), SB250 (*dotted green*) or SB0 (*bold red*) buffers; SEC runs were performed using RB0 buffer as mobile phase. (**C**) Samples were prepared with X30 8% (m/v; *thin blue*), 2% (m/v; *dotted green*) or 0.8% (m/v; *bold red*) and membranes suspended in SB0 buffer; SEC runs were performed using RB0 buffer as mobile phase.

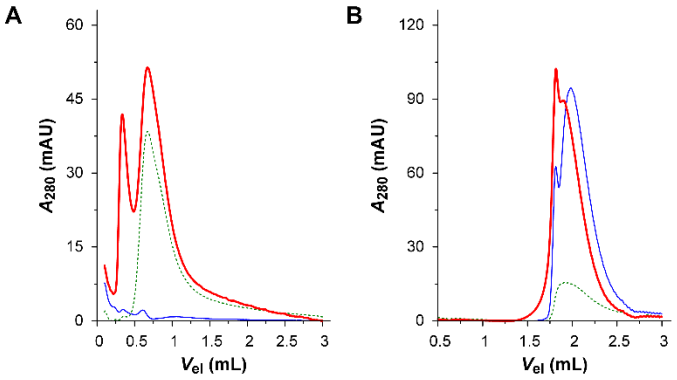

Figure S9. Average chromatograms (280 nm; n = 2) for the IMAC tests on the interference of SMAc co-polymers with the binding of His-tagged proteins on Ni–NTA resins. Samples were prepared in KP100 buffer (pH 8.0); loading was performed in KP100 buffer (pH 8.0), elution was performed with EB buffer. (**A**) Loading of *Cr*GSNOR (0.3%, m/v; *thin blue*), X30 (1%, m/v; *dotted green*) and *Cr*GSNOR/X30 mixture (0.3%/1%, m/v; *bold red*) onto the IMAC column. (**B**) Elution of *Cr*GSNOR (0.3%, m/v; *thin blue*), X30 (1%, m/v; *dotted green*) and *Cr*GSNOR/X30 mixture (0.3%/1%, m/v; *bold red*) from the IMAC column.

Table S1. Total peak areas at 280 nm (expressed in mAU mL) of the chromatograms for the IMAC tests on the interference of SMAc co-polymers with the binding of His-tagged proteins on Ni–NTA resins.

	***Cr*GSNOR (0.3%, m/v)**	**X30 (1%, m/v)**	**Sum**	***Cr*GSNOR/X30 mixture (0.3%/1%, m/v)**	**Difference**	**Estimated loss of His-tagged protein**
Loading (0.23–3 mL)	1.636 (3.8%)	20.666 (70.8%)	22.302	34.225	11.923	27.9%
Elution (1.3–3 mL)	41.032 (96.2%)	8.519 (29.2%)	49.551	38.400	−11.150	26.1%
Total	42.668 (100.0%)	29.185 (100.0%)	71.853	72.626		