Supplementary for Next generation Dried Blood Spot samplers for protein analysis.

Describing trypsin modified smart sampling paper.

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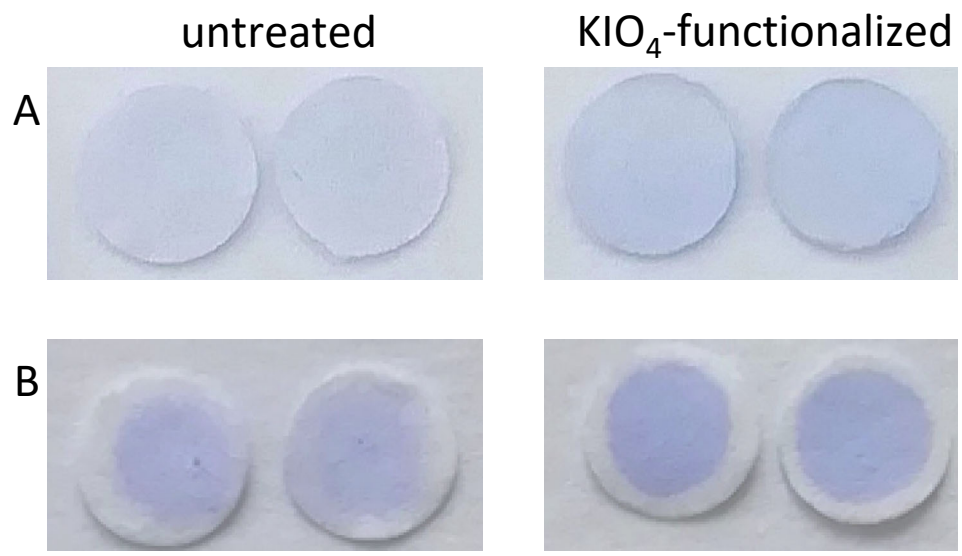

Figure S1: The discs on top (A) are all immobilized using 200 µL 1 % BSA. The discs on the bottom (B) are all immobilized using 0.5 µL 0.5 % BSA. The four discs on the left are untreated (non-functionalized) while the four discs on the right are KIO_4 functionalized. In immobilization procedure used in A the whole disc is covered with BSA solution, in immobilization procedure used in B only 4 - 5 mm of the disc is covered with BSA. After washing with 50 mM ABC the remainder of the BSA is stained. (n=2)

There is visually a slight more intense colour on the functionalized discs on top compared with the untreated discs on top. However, it is challenging to evaluate this difference.

This visual evaluation is easier for the discs in the B row: although the untreated discs are coloured, the circular application spot is less visible. Besides the fact that BSA probably binds by adsorption, it also is washed off as well as it also has spread through the disc. The KIO_4 functionalized discs in the B row show the distinct spot with minimal loss of BSA.

This shows that the modified staining method easily can be used to evaluate protein binding to the discs.

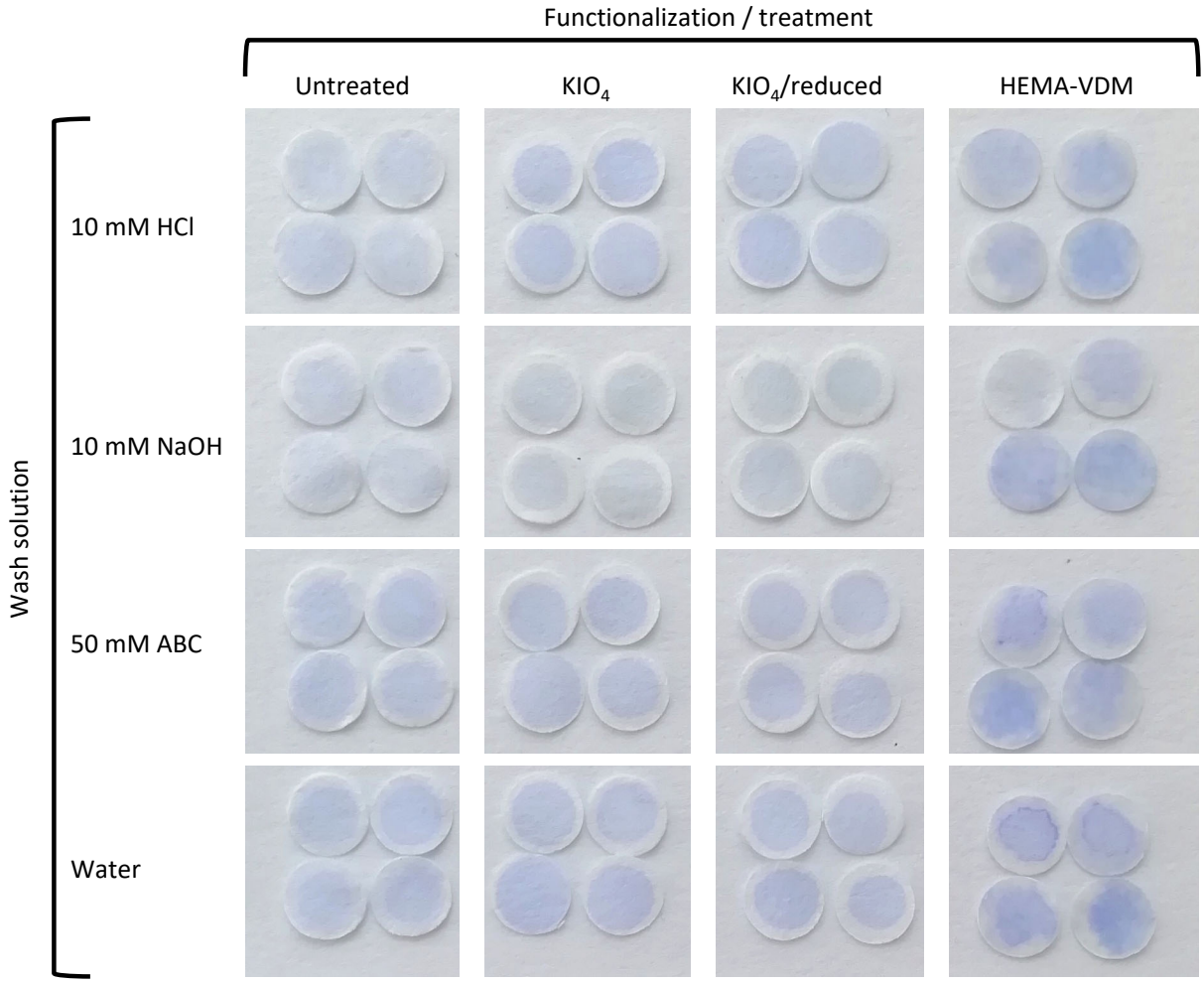

Figure S2A: Stained discs after immobilization of 0.5 µL 0.5% BSA on paper and wash with several solutions (n=4).

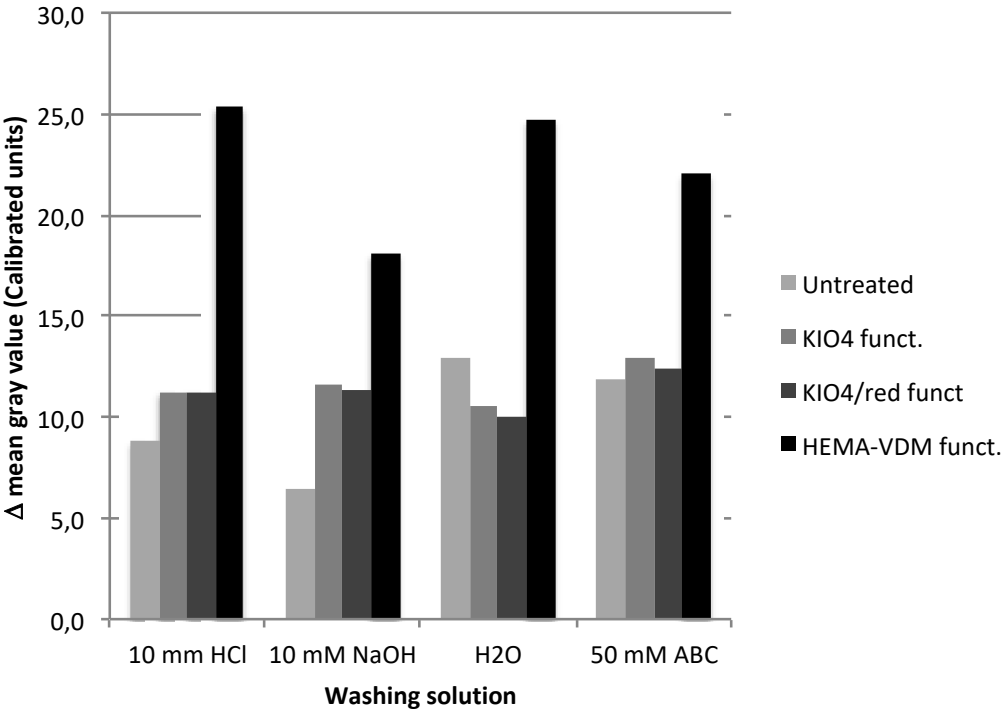

Figure S2B: Δ mean gray values of the discs in figure S2A. Mean gray value of each condition (n=4) is subtracted from the background.

Both 10 mM NaOH and 10 mM HCl appear to be strong washing solutions. HEMA-VDM is most stained, probably binding most protein. Another observation is that the spots on the KIO_4 and KIO_4/reduced discs are circular whereas the spots on the HEMA-VDM discs are more irregular and the colour is not evenly distributed.

Table S1: BAEE measurements: Average ΔA_{253}/min (n=3). In all cases 10 µL 0.1% trypsin is used. For the in-solution measurements 10 µL of the freshly prepared 0.1% trypsin solution, 10 µL of 0.1% trypsin solution placed on a bench for 3 hours or 10 µL of 0.1% trypsin solution placed on a bench overnight is added to the directly to the substrate buffer. For the paper measurements, the discs were extracted in the substrate buffer as described in the experimental section. In all cases the assay was started by adding the substrate (BAEE).

	freshly prepared	3 hour immobilization	overnight immobilization
	Average ΔA_{253}/min (n=3)		
in-solution	0.115	0.114	0.095
untreated paper	n/a	0.031 (72.8%)*	0.025 (73.7%)*
HEMA-VDM funct. paper	n/a	0.026 (77.2%)*	n/a
KIO4 funct. paper	n/a	n/a	0.011 (88.4%)*
*KIO4/reduced funct. paper***	*n/a*	*n/a*	*0.000*

* (estimated yield) based on the activity. Calculated as follows:

((Average $\Delta A_{253}/min_{in\text{-}solution}$ - Average $\Delta A_{253}/min_{paper}$)/(Average $\Delta A_{253}/min_{in\text{-}solution}$)*100%)

** these data were not taken into account in yield estimation since they were not directly comparable with the other conditions.

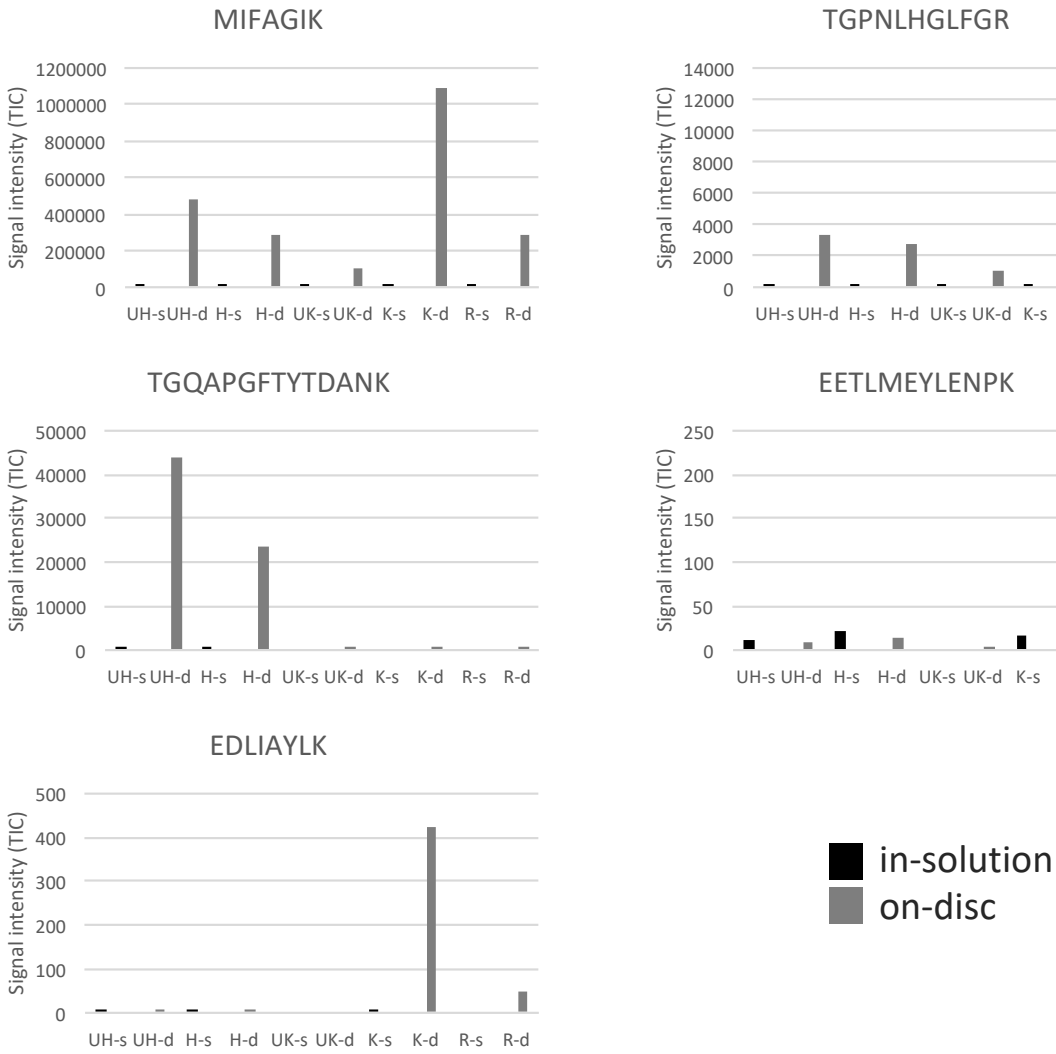

Figure S3: Signal intensities of MIFAGIK, TGPNLHGLFGR, TGQAPGFTYTDANK, ETTLMEYLENPK and EDLIAYLK. Note: the Y axes have different scales.

UH: untreated paper, trypsin immobilized for 3h

H: HEMA-VDM functionalized paper, trypsin immobilized for 3h

UK: untreated paper, trypsin immobilization o/n

K: KIO_4 functionalized paper, trypsin immobilization o/n

R: KIO_4 functionalized paper, trypsin immobilization o/n followed by reduction

-s: in-solution digestion caused by unbound trypsin

-d: on-disc digestion.

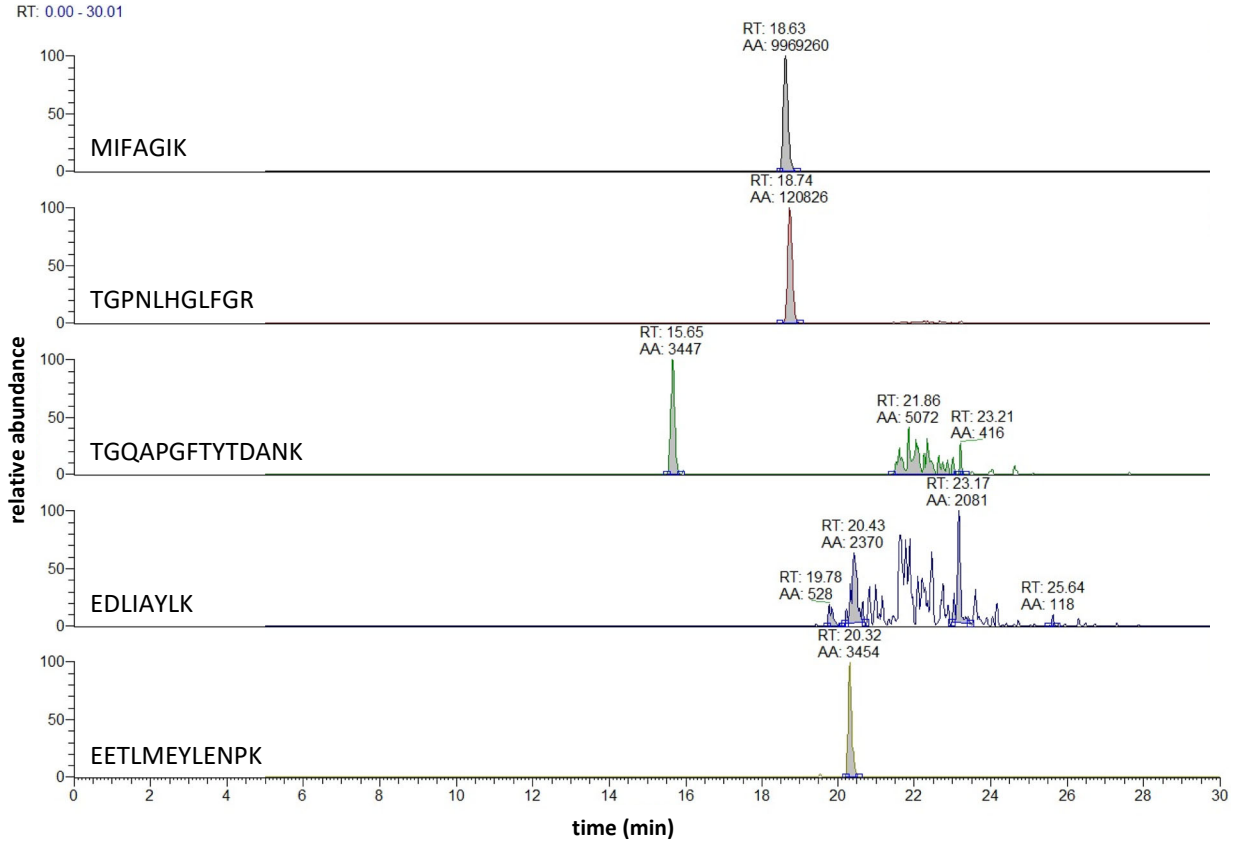

Figure S4: Trypsin activity on-disc. Production of five “zero missed-cleaved” peptides of Cytochrome C after 10 minutes incubation. The *m*/*z* values of the traces measured are shown in table 1. Ten µL 10 mg/mL CytC applied on a dry KIO_4 functionalized trypsin disc which earlier was washed extensively eight times with 2 mL 0.05% Tween followed by 2 mL water and concluded with 100 µL 50 mM ABC. The disc was kept in a sealed container for 10 min. After the seal was taken off the discs were extracted in 110 µL 0.1% formic acid. Before the analysis the extract was diluted 10 x in 0.1 % FA.