Supporting Information

SPRi-MALDI MS: characterization and identification of a kinase from cell lysate by specific interaction with different Designed Ankyrin Repeat Proteins

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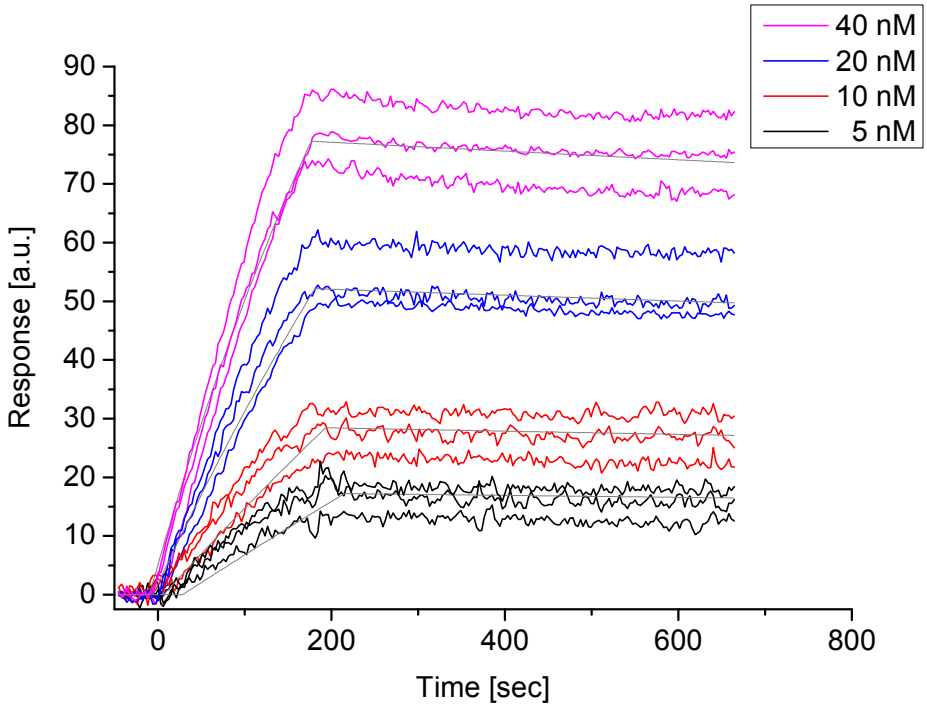

Fig. S - 1 SPRi measurement for 276_C08 with a concentration series of RPS6KA2. K_D value of 0.58 ± 0.01 nM was calculated (fitted curves shown in grey).

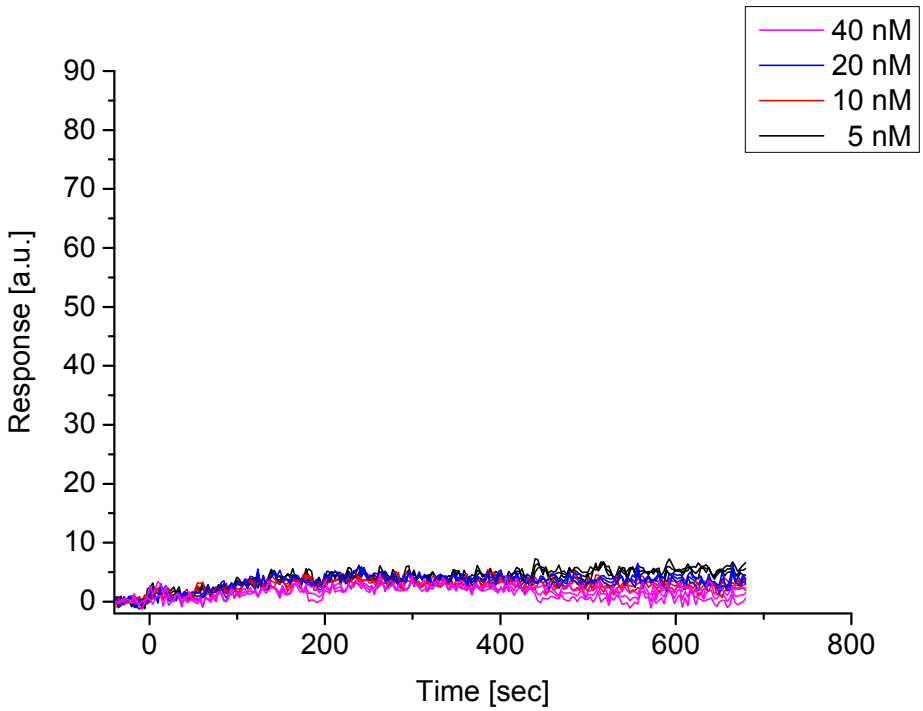

Fig. S - 2 SPRi measurement for 244_A07 with a concentration series of RPS6KA2.

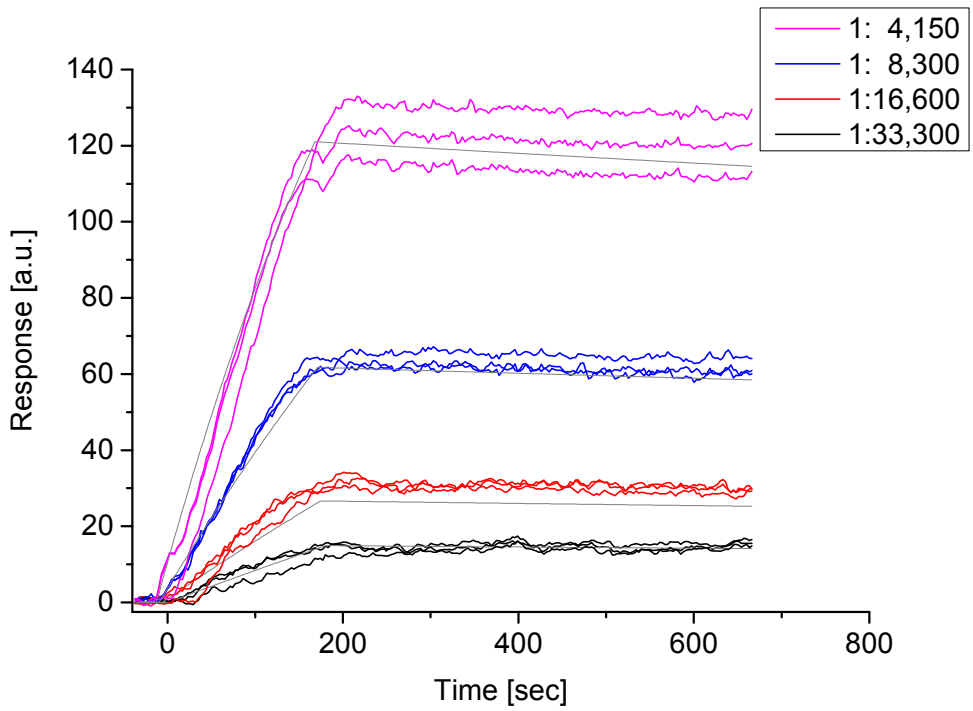

Fig. S - 3 SPRi measurement for 113_D02 with a concentration series of SH-SY5Y. K_D value of 3.6 ± 0.2 nM was calculated (fitted curves shown in grey).

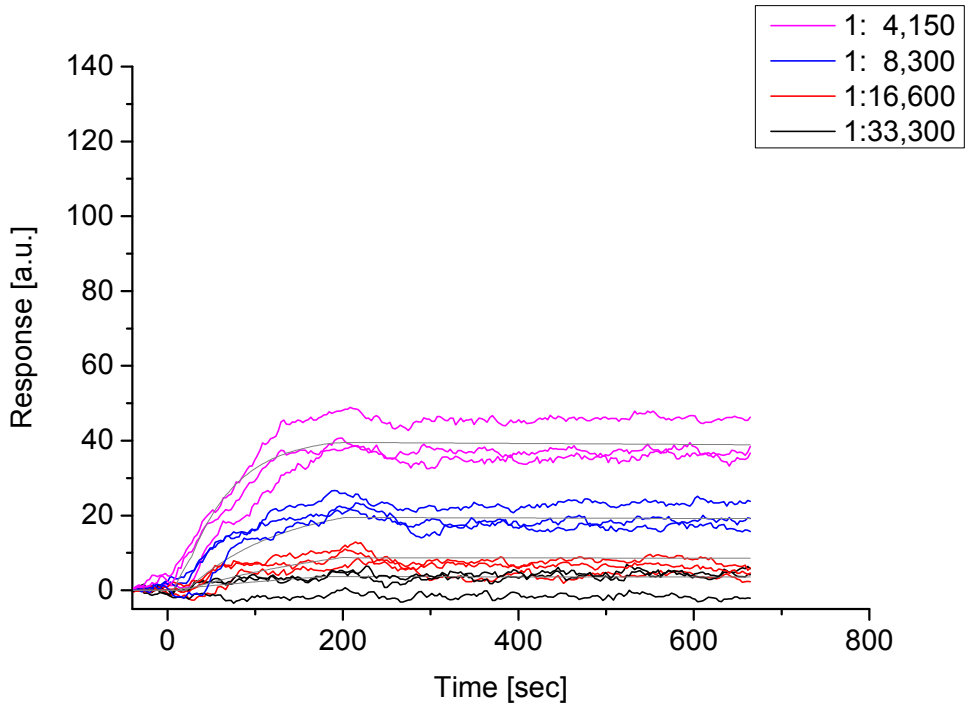

Fig. S - 4 SPRi measurement for 276_C08 with a concentration series of SH-SY5Y. K_D value of 1.64 ± 0.05 nM was calculated (fitted curves shown in grey).

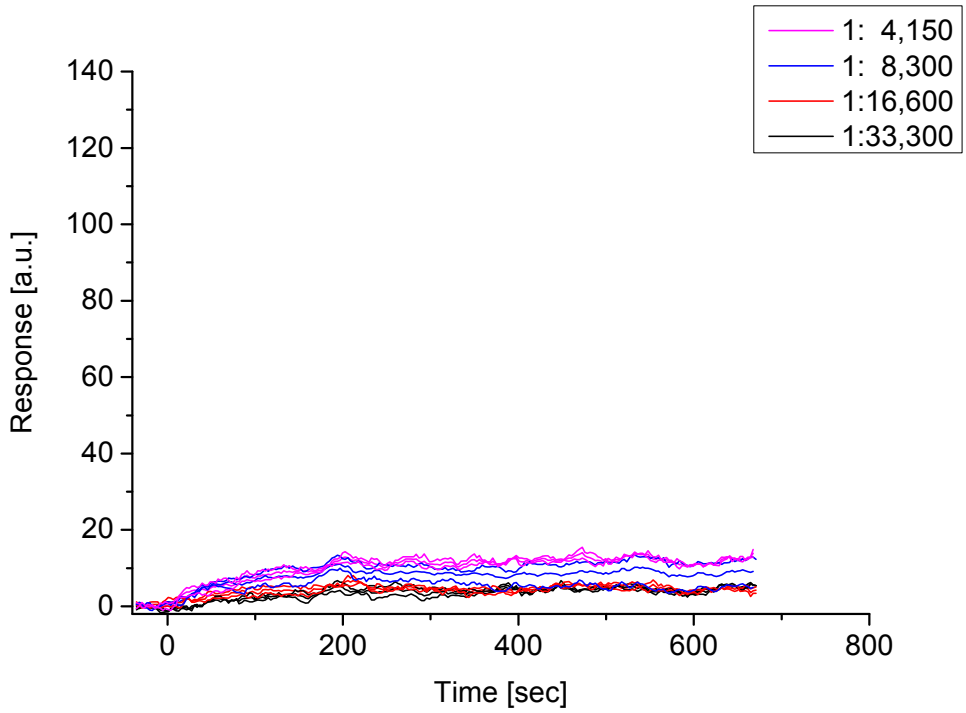

Fig. S - 5 SPRi measurement for 244_A07 with a concentration series of SH-SY5Y.

Table S - 1 Association and dissociation rate constants and K_Ds for several DARPins interacting with the target protein RPS6KA2 from the cell lysate SH-SY5Y. Standard deviations reflect measurements on triplicates of the immobilized DARPin and global fitting.

DARPin	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D
243_C05	$1.38 \pm 0.04 \times 10^5$	$5.90 \pm 0.20 \times 10^{-5}$	0.43 nM (±0.01 nM)
276_C08	$1.29 \pm 0.04 \times 10^5$	$2.13 \pm 0.04 \times 10^{-4}$	1.64 nM (±0.05 nM)
113_D02	$2.10 \pm 0.10 \times 10^4$	$7.50 \pm 0.20 \times 10^{-5}$	3.60 nM (±0.02 nM)
244_A07	-	-	-

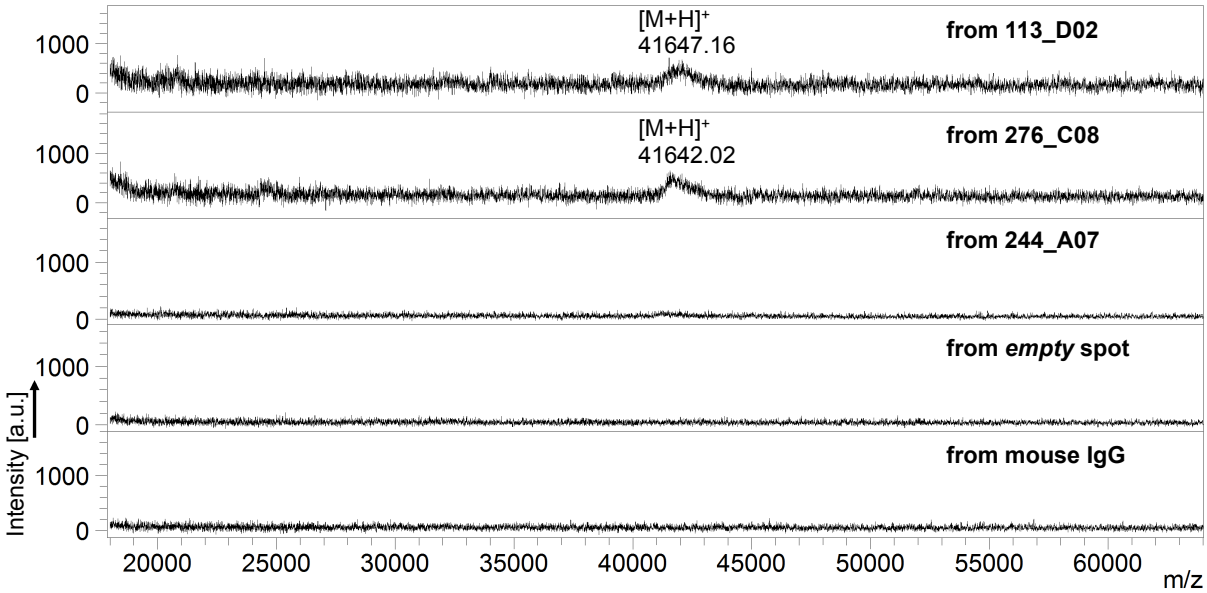

Fig. S - 6 On-chip MALDI mass spectra of intact RPS6KA2 captured on the SPRi gold slide. The two DARPins 113_D02 and 276_C08 showed similar results as 243_C05 (Fig. 3). RPS6KA2 signals were not detectable in the negative control (mouse IgG), nor on empty spots (no immobilized protein), nor on the 244_A07 spot, which showed also no SPR signal (Fig. S – 5).

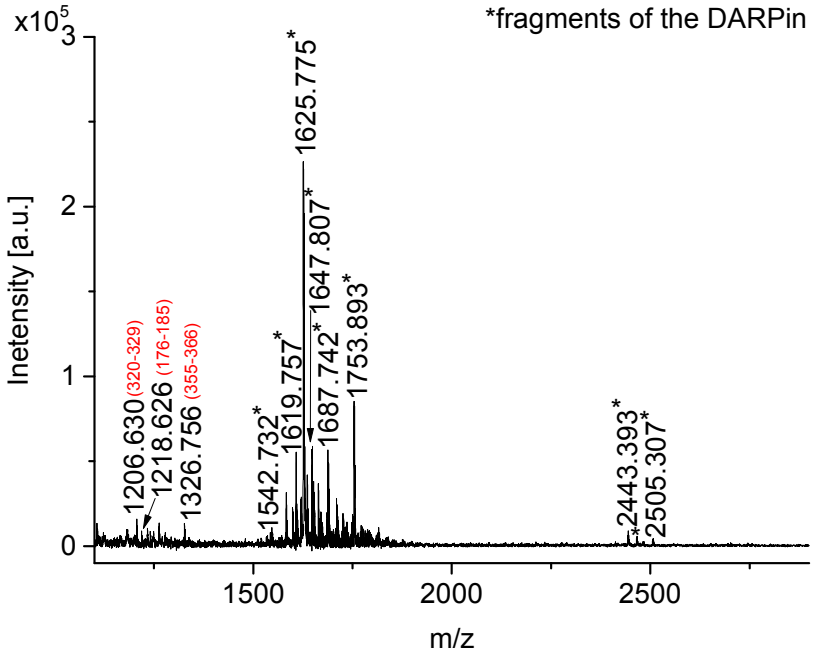

Fig. S - 7 On-chip MALDI mass spectrum after SPRi measurement and on-chip proteolysis of captured RPS6KA2 retained on 113_D02. Fragments of RPS6KA2 are labeled with its sequence range and peptides of the DARPin 113_D02 are marked with *. Three fragments identified, 4.4% sequence coverage, 6.8% intensity coverage, Mascot score: 31

Table S - 2 Peptide mass fingerprint of on-chip digested captured RPS6KA2 retained on 113_D02 after SPRi measurement (*fragments of the DARPin 113_D02)

Meas. m/z	Theor. m/z	|Δ ppm|	Seq. range	Sequence
1,206.645	1,206.611	28.2	320-329	EYLSPNQLSR
1,218.694	1,218.684	8.2	176-185	DLKPSNILYR
1,326.757	1,326.738	14.3	355-366	LEPVLSSNLAQR
1,542.732	1,542.706	13.0	3-16	GSHHHHHHGSDLGK*
1,619.757	1,619.752	3.1	32-46	ILMANGADVNAMDER*
1,625.775	1,625.781	3.7	148-167	LLEAAFYGQDDEVR*
(1,647.807)	(1,647.763)	(24.7)		(Na^+-adduct)
(1,687.742)	(1,687.702)	(23.7)		(Cu^{2+}-adduct)
1,753.893	1,753.876	9.7	17-31	KLLEAAFYGQDDEVR*
2,443.393	2,443.334	24.1	47-68	GWTPLHLAASQGHLEIVEVLLK*
				(W double oxidized)
(2,465.393)	(2,465.316)	(26.8)		(Na^+-adduct)
(2,505.307)	(2,505.256)	(20.4)		(Cu^{2+}-adduct)

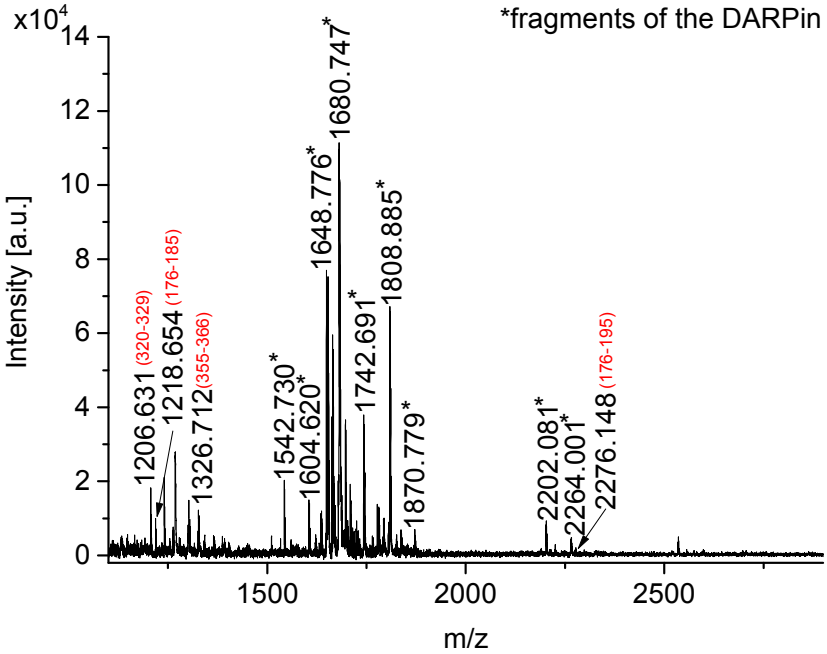

Fig. S - 8 On-chip MALDI mass spectrum after SPRi measurement and on-chip proteolysis of captured RPS6KA2 retained on 276_C08. Fragments of RPS6KA2 are labeled with its sequence range and peptides of the DARPin 276_C08 are marked with *. Four fragments identified, 7.1% sequence coverage, 8.3% intensity coverage, Mascot score: 54

Table S - 3 Peptide mass fingerprint of on-chip digested captured RPS6KA2 retained on 276_C08 after SPRi measurement (*fragments of the DARPin 276_C08)

Meas. m/z	Theor. m/z	|Δ ppm|	Seq. range	Sequence
1,206.613	1,206.611	1.7	320-329	EYLSPNQLSR
1,218.654	1,218.684	24.6	176-185	DLKPSNILYR
1,326.712	1,326.738	19.6	355-366	LEPVLSSNLAQR
2,276.148	2,276.158	4.4	176-195	DLKPSNILYRDESGSPESIR
1,542.730	1,542.706	15.6	3-16	GSHHHHHHGSDLGK*
(1,604.620)	(1,604.627)	(1.9)		(Cu^{2+}-adduct)
1,648.776	1,648.797	12.7	18-31	LLEAAWFGQDDEVR*
1,680.747	1,680.786	23.2	18-31	LLEAAWFGQDDEVR *
				(W doubly oxidized)
(1,742.691)	(1,742.708)	(9.8)		(Cu^{2+}-adduct)
1,808.885	1,808.881	2.2	17-31	KLLEAAWFGQDDEVR*
				(W doubly oxidized)
(1,870.779)	(1,870.803)	(12.8)		(Cu^{2+}-adduct)
2,202.081	2,202.092	5.0	148-167	TPFDLAIDNGNEDIAEVLQK*
(2,264.001)	(2,264.014)	(5.7)		(Cu^{2+}-adduct)

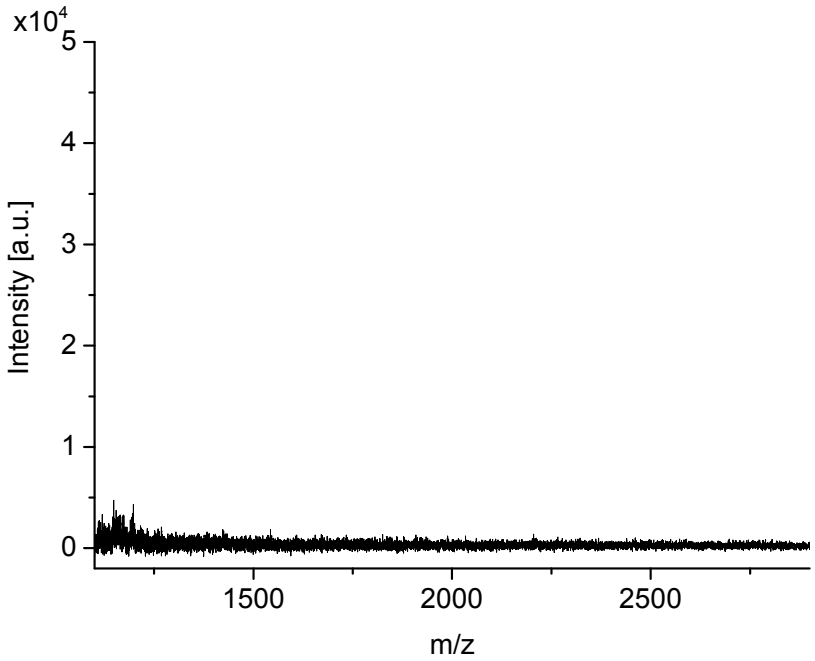

Fig. S - 9 On-chip MALDI mass spectrum after SPRi measurement and on-chip proteolysis of "captured" RPS6KA2 retained on 244_A07. No RPS6KA2 fragments detectable due to no specific interaction with 244_A07.

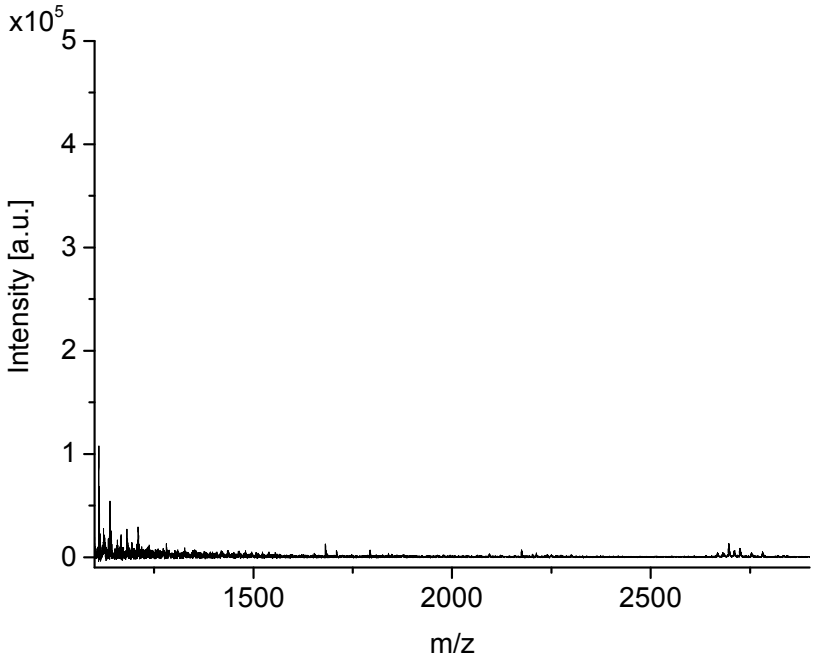

Fig. S - 10 On-chip MALDI mass spectrum after SPRi measurement and on-chip proteolysis of "captured" RPS6KA2 retained on an empty spot (as negative control). No RPS6KA2 fragments detectable due to no specific interaction.

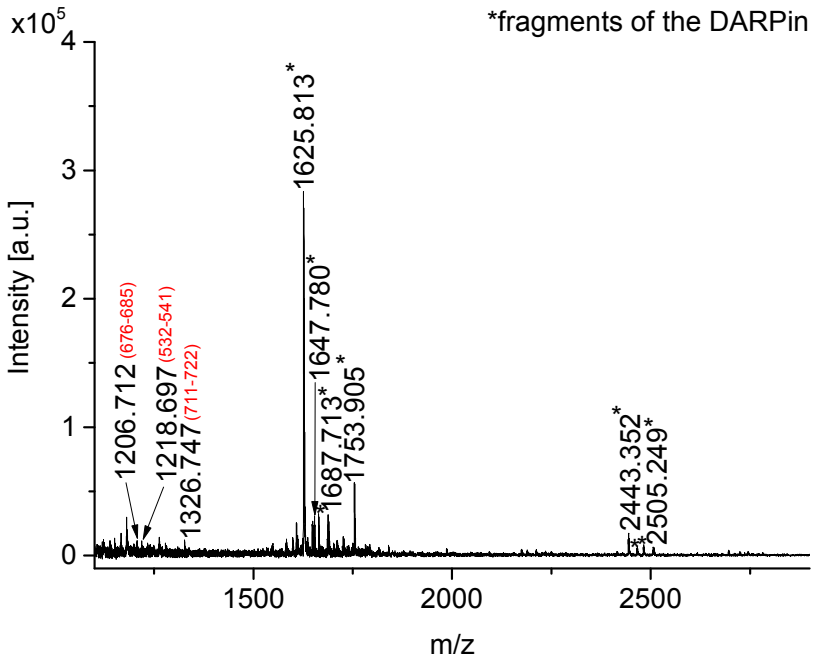

Fig. S - 11 On-chip MALDI mass spectrum after SPRi measurement and on-chip tryptic digestion of captured RPS6KA2 (from SH-SY5Y) retained on 113_D02. Fragments of RPS6KA2 are labeled with its sequence range and peptides of the DARPin 113_D02 are marked with *. Three fragments identified, 4.4% sequence coverage, 5.4% intensity coverage, Mascot score: 30

Table S - 4 Peptide mass fingerprint of on-chip digested captured RPS6KA2 (from SH-SY5Y) retained on 243_C05 after SPRi measurement (*fragments of the DARPin 243_C05)

Meas. m/z	Theor. m/z	|Δ ppm|	Seq. range	Sequence
1,206.645	1,206.611	28.2	676-685	EYLSPNQLSR
1,218.694	1,218.684	8.2	532-541	DLKPSNILYR
1,326.757	1,326.738	14.3	711-722	LEPVLSSNLAQR
1,625.713	1,625.781	19.7	148-167	LLEAAFYGQDDEVR*
(1,647.780)	(1,647.763)	(10.3)		(Na^+-adduct)
(1,663.781)	(1,663.736)	(27.0)		(K^+-adduct)
(1,687.713)	(1,687.702)	(6.5)		(Cu^{2+}-adduct)
1,753.905	1,753.876	16.5	17-31	KLLEAAFYGQDDEVR*
2,443.386	2,443.334	21.3	47-68	GWTPLHLAASQGHLEIVEVLLK*
				(W doubly oxidized)
(2,465.399)	(2,465.316)	(33.7)		(Na^+-adduct)
(2,481.371)	(2,481.290)	(32.6)		(K^+-adduct)
(2,505.270)	(2,505.256)	(5.6)		(Cu^{2+}-adduct)

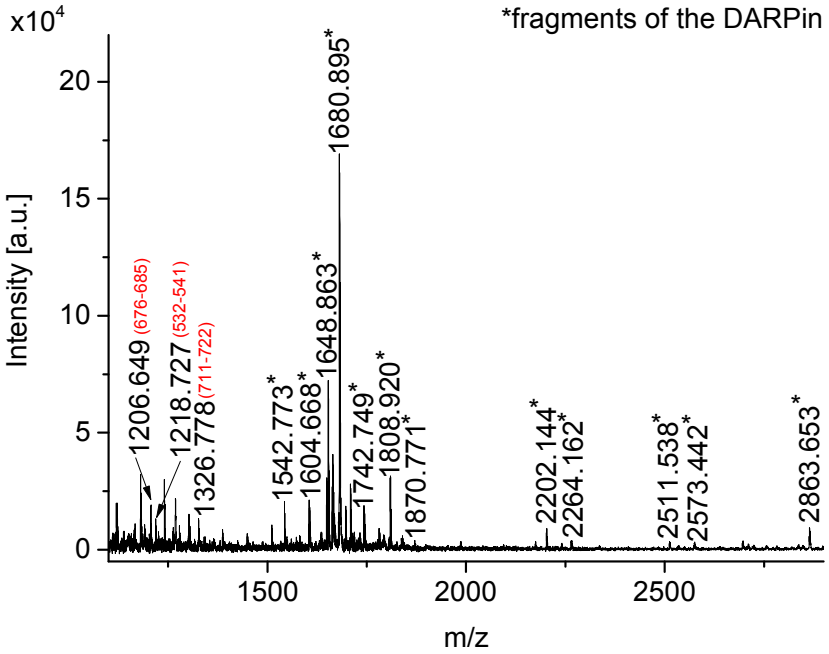

Fig. S - 12 On-chip MALDI mass spectrum after SPRi measurement and on-chip tryptic digestion of captured RPS6KA2 (from SH-SY5Y) retained on 276_C08. Fragments of RPS6KA2 are labeled with its sequence range and peptides of the DARPin 276_C08 are marked with *. Three fragments identified, 4.4% sequence coverage, 7.1% intensity coverage, Mascot score: 56

Table S - 5 Peptide mass fingerprint of on-chip digested captured RPS6KA2 retained on 276_C08 after SPRi measurement (*fragments of the DARPin 276_C08)

Meas. m/z	Theor. m/z	|Δ ppm|	Seq. range	Sequence
1,206.649	1,206.611	31.5	676-685	EYLSPNQLSR
1,218.727	1,218.684	35.3	532-541	DLKPSNILYR
1,326.778	1,326.738	30.1	711-722	LEPVLSSNLAQR
1,542.730	1,542.706	43.4	3-16	GSHHHHHHGSDLGK*
(1,604.620)	(1,604.627)	(51.7)		(Cu^{2+}-adduct)
1,648.863	1,648.797	40.0	18-31	LLEAAWFGQDDEVR*
1,680.895	1,680.786	64.9	18-31	LLEAAWFGQDDEVR *
				(W double oxidized)
(1,742.749)	(1,742.708)	(23.5)		(Cu^{2+}-adduct)
1,808.957	1,808.881	42.0	17-31	KLLEAAWFGQDDEVR*
				(W doubly oxidized)
(1,870.771)	(1,870.803)	(17.1)		(Cu^{2+}-adduct)
2,202.178	2,202.092	39.1	148-167	TPFDLAIDNGNEDIAEVLQK*
(2,264.162)	(2,264.014)	(65.4)		(Cu^{2+}-adduct)
2,511.538	2,511.433	41.8	112-134	AGITPLHLAAVWGHLEIVEVLLK*
				(W doubly oxidized)
(2,573.442)	(2,573.355)	(33.8)		(Cu^{2+}-adduct)
2,863.653	2,863.525	(44.7)	77-101	DVFGWTPLHLAAHHGHLEIVEVLLK*
				(W doubly oxidized)

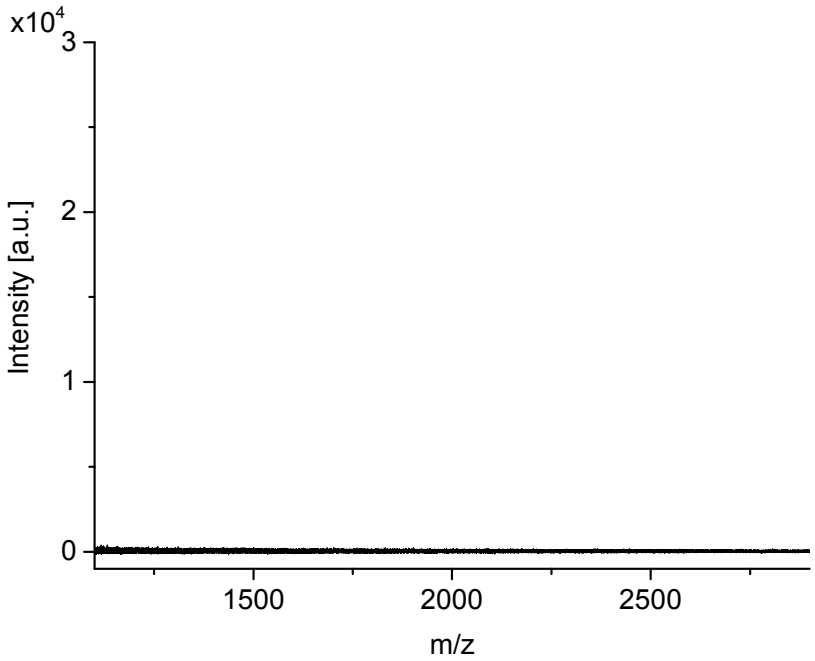

Fig. S - 13 Negative control: on-chip MALDI mass spectrum after SPRi measurement and on-chip tryptic digestion of "captured" RPS6KA2 (from SH-SY5Y) retained on 244_A07. No RPS6KA2 fragments detectable due to the absence of any specific interaction.

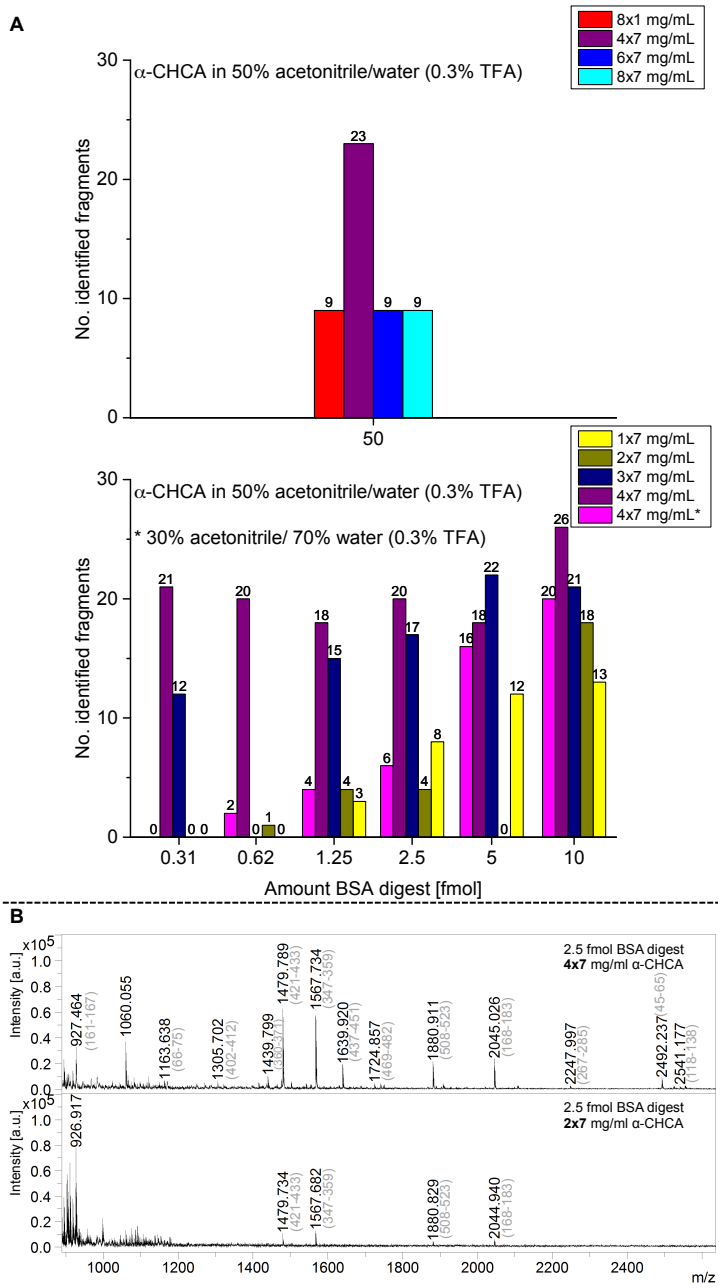

Fig. S - 14 Preliminary optimization studies for matrix application with commercially available tryptic digest of BSA on a stainless steel target. A: Two different concentrations of α-CHCA (1 mg/mL vs. 7 mg/mL) and different numbers of spray cycles were investigated (upper graph). 8x1 mg/mL means that 1 mL aliquots of a 1 mg/mL α-CHCA solution were sprayed eight times. In addition it was tested if lower amounts than 4x7 mg/mL of matrix or if a higher water content in the solvent increase the number of identified BSA fragments (lower graph). B: Comparison between the resulting mass spectra with the best matrix condition (4x7 mg/mL α-CHCA in 50% acetonitrile/water (0.3% TFA)) and a poor matrix condition (2x7 mg/mL). For a better overview not all identified BSA fragments for 4x7 mg/mL α-CHCA were labeled (grey numbers in brackets represent the sequence range of the peptide; overall 20 fragments were identified). It is obvious that with lower numbers of identified fragments also their intensities are decreasing.

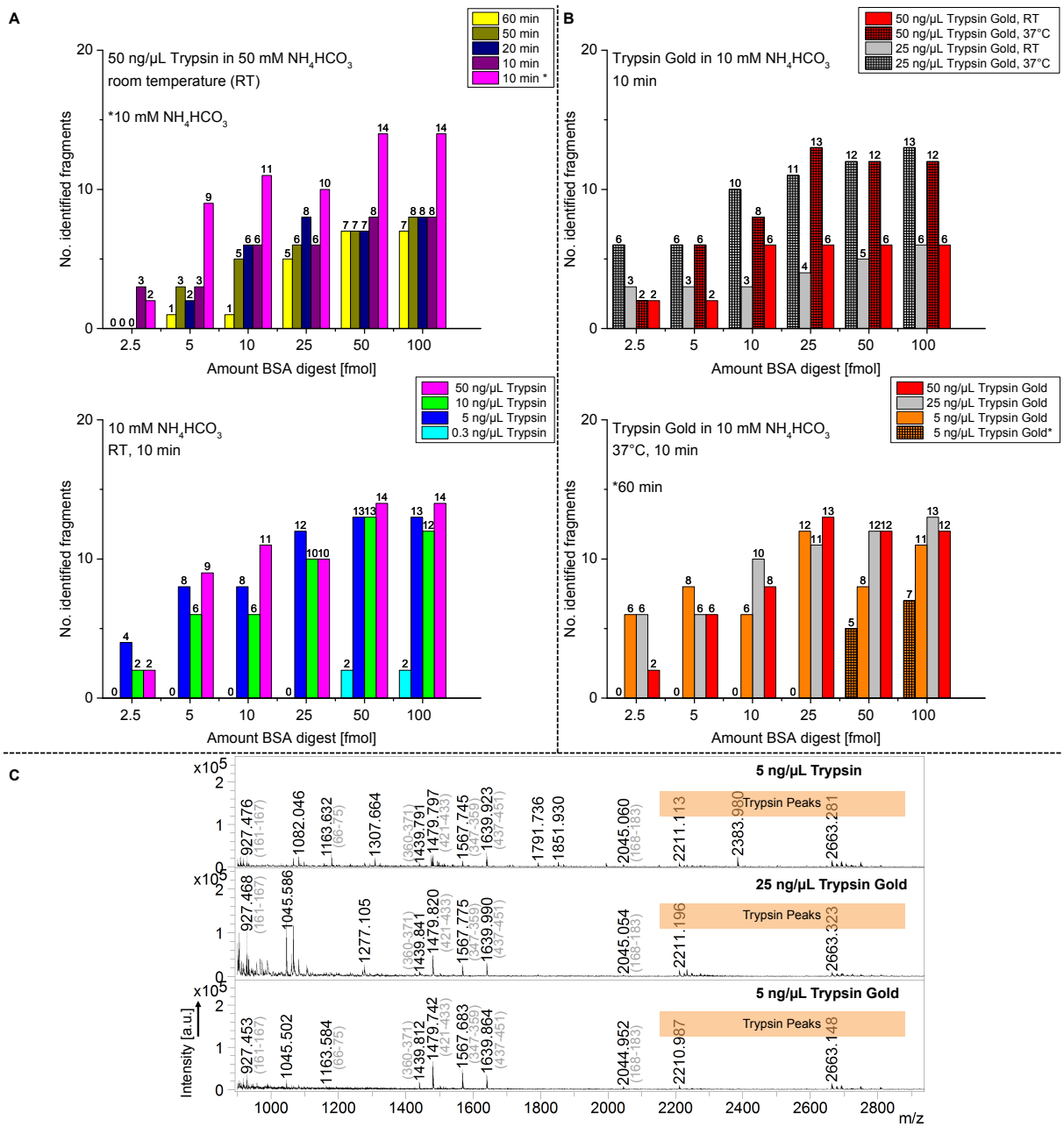

Fig. S - 15 Preliminary optimization studies for tryptic digestion with BSA on a stainless steel target. A: Influence of incubation time, salt content of NH_4HCO_3 (both at pH 8.5) and concentration of trypsin. The difference between 50 ng/µL and 5 ng/µL trypsin was not very big but intensities of autolysis fragments decreased enormously. However autolysis fragments were still at a similar intensity level as of BSA fragments (C upper spectrum). B: Trypsin Gold, which is methylated and supposed to have reduced autolysis properties, was investigated towards temperature, concentration and incubation time. Trypsin Gold was most effective after 10 min at 37°C. C: Comparison between the resulting mass spectra at 5 fmol BSA. Eight fragments were identified with 5 ng/µL trypsin (upper spectrum, 1193.673 m/z (25-34) not labeled) and 5 ng/µL Trypsin Gold (lower spectrum, 1511.792 m/z (438-451) not labeled), respectively. Grey numbers in brackets represent the sequence range of the peptide. However the intensity of autolysis fragments is much lower than for BSA fragments with Trypsin Gold, and this trypsin version was therefore chosen for further experiments.