Supplementary information

Supplementary methods

Proteomic Identification of Cleavage Sites (PICS):

Identification of protease cleavage sites was performed with minor changes according to PICS protocols described by Schilling et al. [1]. Three different PICS libraries were generated, namely **h**uman **e**mbryonic **k**idney (**HEK**) (ATCC-Number CRL-1573, ATCC, Manassas, VA,) cell based, *Escherichia coli (E. coli)* (Thermo Fisher Scientific, Waltham, USA) ^{14}N and *E. coli* ^{15}N.

HEK-293 cells were cultured in dulbecco's modified eagle's medium (10 % fetal bovine serum; 1 % penicillin-streptomycin) and grown to confluence. Upon harvesting, cells were washed with cold **p**hosphate **b**uffered **s**aline (**PBS**), centrifuged at 1500 g / 4 °C for 10 min. The cell pellet was retained and washed twice with PBS.

E. coli (DH5α) cells were cultured either in lysogeny broth medium (*E. coli* ^{14}N) or Spectra 9 medium (Eurisotop, CGM-3030) (*E. coli* ^{15}N) for 16 h at 37 °C. The cell suspension was centrifuged at 4000 g / 4 °C for 20 min. The cell pellet was retained and washed two times with PBS.

All cells were lysed by suspension in lysis buffer (125 mM tris(hydroxymethyl)aminomethane (Tris), 5 % **s**odium **d**odecyl **s**ulfate (**SDS**), 100 mM **di**thio**t**hreitol (**DTT**), 1 mM **p**henyl**m**ethyl**s**ulfonyl **f**luoride (**PMSF**)) followed by three cycles of sonication (Sonopuls HD3100, Bandelin, Berlin, Germany) with 25 % amplitude, 30 s sonication, 90 s pause for 10 min on ice. After sonication, an equal amount of radio-immunoprecipitation-assay-buffer (incl. 6 M urea, 5% SDS) was added, shaken for 90 min at 4 °C and finally centrifuged at 20,000 g for 30 min. Protein concentration in the supernatant was determined via **b**icin**c**honinic **a**cid assay (**BCA**).

The proteins were alkylated as described. Briefly, lysates were treated with 20 mM **i**odo**a**cet**a**mide (**IAA**) in 100 mM 4-(2-**h**ydroxy**e**thyl)-1-**p**iperazine**e**thane**s**ulfonic acid (**HEPES**), pH 7.5 for 1 h in the dark; IAA was quenched with 5 mM DTT. Precipitation was performed with 15 % **t**ri**c**hlor**a**cetic acid (**TCA**) solution

(4 °C, 1h) followed by centrifugation at 8,500 g for 1 hour, 4 °C. The pellets were washed twice with cold methanol and air-dried. Proteins were solubilized in ice-cold 20 mM NaOH in an ultrasonic bath for 1 h followed by tip sonication as mentioned above. Samples were adjusted to a final concentration of 2 g/L in 200 mM HEPES, pH 7.5, centrifuged again for 30 min at 8,500 g and the supernatant was retained.

To generate peptide libraries, lysates were digested with trypsin (pH 8.5, 1:100 protease, 16 h 37 °C). Digestion was verified via SDS gel, with no major bands >10 kDa. Trypsin was heat inactivated at 70 °C for 20 min followed by the addition PMSF to a final concentration of 1 mM. Libraries were adjusted to 1 M guanidine hydrochloride and centrifuged at 4500 rpm, at 4 °C for 1 h; the supernatant was retained.

Peptides were alkylated again: Incubation with 5 mM DTT for 1 h at 37 °, was followed by addition of 40 mM IAA and incubation for 1.5 h at 37° in the dark. IAA was quenched with 15 mM DTT and incubated at 37 °C for 10 min.

Chemical modification was performed as described [1]. Briefly, 30 mM formaldehyde and 30 mM sodium cyanoborohydride were added and allowed to react at 25 °C for 2 h. This step was repeated once for 16 h. After that, 100 mM glycine was added and incubated at 25 °C for 30 min. The sample was acidified with TFA (0.5%) and degassed. All samples were purified by C18 solid phase extraction and lyophilized. The lyophilised samples were resolved in 200 mM HEPES, pH 7.5, final concentrations were adjusted to 2 g/L. Aliquots were prepared and stored at -80 °C until further usage.

MMP-8 PICS:

300 µg of peptide libraries were adjusted to 200 mM NaCl, 50 mM HEPES, pH 7.5, 5 mM $CaCl_2$ and 1µM $ZnCl_2$. The peptide libraries were incubated with 5 µg activated MMP-8 (see below), for 16 h at 37 °C.

MMP-8 specific peptide fragments were enriched, following the published protocols [1]. Briefly, the sample was adjusted to pH 7-8, 0.5 mM sulfo-NHS-SS-biotin were added and incubated for 2 h at 25 °C. Biotin-tagged peptide fragments were enriched on high-capacity streptavidin sepharose. Washing and elution was done on spin columns. Removal of non-specifically bound peptides was achieved by washing nine times

with wash buffer (50 mM HEPES, pH 7.5, 150 mM NaCl). Target peptides were eluted with two times 500 µL elution buffer (50 mM HEPES, 10 mM DTT, pH 7.5), incubated for 2 h at 25 °C. The column was centrifuged and the flow-through was applied to the resin one more time. Afterwards the samples were purified via solid phase extraction (StrataX, Phenomenex) (Washed with 4-5 mL of 0.4 % **f**ormic **a**cid (**FA**), elution in 1.8 mL 0.4 FA/80 % **ac**eto**n**itrile **ACN**) and stored at -80 °C until analysis.

Liquid chromatography – mass spectrometry (LC-MS) / Data Analysis:

Samples were resuspended in loading buffer (0,2 % FA/2 % ACN/98 % H_2O) prior to analysis. NanoLC-MS/MS analyses were performed on an LTQ-Orbitrap Velos Pro (Thermo Scientific) equipped with a PicoView Ion Source (New Objective) and coupled to an EASY-nLC 1000 (Thermo Scientific). Peptides were loaded on capillary columns (PicoFrit, 30 cm x 150 µm ID, New Objective) self-packed with ReproSil-Pur 120 C18-AQ, 1.9 µm (Dr. Maisch, Ammerbuch-Entringen, Germany) and separated with a 120-minute linear gradient from 3 % to 30 % acetonitrile and 0.1 % formic acid and a flow rate of 500 nL/min.

MS scans were acquired in the Orbitrap analyzer with a resolution of 30000 at m/z 400, MS/MS scans were acquired in the Orbitrap analyzer with a resolution of 7500 m/z at 400 using HCD fragmentation with 30 % normalized collision energy. A TOP5 data-dependent MS/MS method was used; dynamic exclusion was applied with a repeat count of 1 and an; singly charged precursors were excluded from selection. Minimum signal threshold for precursor selection was set to 50000. Predictive AGC was used with AGC target a value of 1e6 for MS scans and 5e4 for MS/MS scans. Lock mass option was applied for internal calibration in all runs using background ions from protonated decamethylcyclopentasiloxane (m/z 371.10124).

Liquid chromatography – long-term storage sample

Analytical HPLC was carried out via an Agilent 1260 infinity II HPLC (Agilent Technologies Inc., Waldbronn, Germany) using a Zorbax 300SB-CN 5µm analytical 4.6 x 150 mm LC column (Agilent Technologies Inc., Waldbronn, Germany) The device was equipped with a variable wavelength dectector (G7114A, Agilent), an automatic vial sampler (G7129C, Agilent), flexible Pump (G7104C, Agilent), and

multicolumn oven (G7116A, Agilent). Mobile phase A was 0.1% TFA in Millipore water. Mobile phase B was ACN with 0.1% TFA, flow was set to 1 ml/min, injection volume was 20 µl, and the wavelength of the detector was set to λ = 214 nm. The gradient was increased in 18 min from 5 to 95 % B, then held at 95 % B for 2 min, reduced to 5 % B within 2 min, and held at 5 % B for 9 min.

Data analysis:

Data analysis was performed using PMi-Byos software V3.11 by Protein Metrics inc. (United states). Datafiles were searched, with the following settings: Protease set as trypsin (C-terminal to R, K), semi-specific digest (ragged peptide N-terminus), up to 3 missed cleavages. Precursor Tolerance of 7.5 ppm, fragment mass tolerance of 15 ppm. Carbamidomethyl @ C as fixed modification, dimethyl/methyl/thioacyl @ K as common and thiacyl @ peptide N-term as common (residue from the cleavable enrichment probe). With max allowed common modifications at 3 and rare max set to 1. For 15N samples a fixed modification for each amino acid was used to enable search of heavy labelled peptides (see list below). (We tested to add modifications for potentially unlabeled, or not fully labelled amino acids (e.g. 15N-remove_Gly / -0,997035 @ G), however due to the needed number of additional modifications - at least 21 and up to 30 to account for partial labeling - we found no improvement in the results generated – Data not shown).

Data was searched against the following databases:

HEK: Human TREMBL+isoforms (downloaded from uniport)

E. coli: *E. coli* Reference proteome (downloaded from uniport)

Search Results were filtered on peptide level to not contain reverse proteins (from decoys) and only contain peptides with a PEP 2D score of <0.1, total score >100 and containing 'N-terminal thioacyl.

The resulting peptide list was submitted to the WEB-PICs application (http://clipserve.clip.ubc.ca/pics) and the results were used to enable design of custom peptides [2].

15N – custom modifications:

15N_Gly / +0.997035 @ G | fixed

15N_Ala / +0.997035 @ A | fixed

15N_Ser / +0.997035 @ S | fixed

15N_Thr / +0.997035 @ T | fixed

15N_Cys / +0.997035 @ C | fixed

15N_Val / +0.997035 @ V | fixed

15N_Leu / +0.997035 @ L | fixed

15N_Ile / +0.997035 @ I | fixed

15N_Met / +0.997035 @ M | fixed

15N_Pro / +0.997035 @ P | fixed

15N_Phe / +0.997035 @ F | fixed

15N_Tyr / +0.997035 @ Y | fixed

15N_Asp / +0.997035 @ D | fixed

15N_Glu / +0.997035 @ E | fixed

15N_Trp / + 1.994070 @ W | fixed

15N_Asn / +1.994070 @ N | fixed

15N_Gln / +1.994070 @ Q | fixed

Peptide synthesis:

All peptides described in this manuscript were synthesized using **f**luorenyl**m**eth**o**xy**c**arbonyl protecting group (**Fmoc**) based solid phase peptide synthesis, following established protocols using a polypropylene-reactor (MultiSynTech, Witten, Germany).

Syntheses were performed on 100 µmol loaded 2-**C**hloro**t**rityl **c**hloride resin (**CTC**-Resin). The respective first amino acid was coupled in 5-molar excess in **d**i**c**hlor**m**ethane (**DCM**) / **d**i**i**so**p**ropyl**e**thyl**a**mine (**DIPEA**) at 25 °C under rigorous shaking for at least 3 hours. Free CTC-binding sites were blocked with

methanol for 30 minutes at 25 °C under rigorous shaking. The resin was washed 3 times with DCM and 3 times with **di****m**ethyl**f**ormamide (**DMF**).

The subsequent amino acids were either coupled via semi-automatic Biotage® Initiator+ SP Wave (Biotage, Uppsala, Sweden) peptide synthesizer (synthesizer 1) or Liberty Blue™ (CEM Corporation, Matthews, USA) automatic peptide synthesizer (synthesizer 2).

Synthesizer 1:

500 µmol of the respective Fmoc-protected amino acid was dissolved in DMF containing 0.5 M hydroxybenzotriazole, 0.5 M N,N′-**Di**isopropyl**c**arbodiimide (**DIC**) and 0.2 M DIPEA at 50 °C for 10 min followed by three wash steps with DMF. Fmoc deprotection was carried out via incubation in 20 % piperidine in DMF for 3 min at 75 °C followed by four wash steps with DMF.

Synthesizer 2:

0.2 M of the respective amino acid, in DMF containing 1 M ethyl cyanohydroxyiminoacetate and 0.5 M DIC for 15 s at 75 °C, followed by 110 s at 90 °C. Fmoc deprotection was carried out in DMF with 20 % piperidine at 75 °C for 15 s followed by 50 s at 90 °C.

Completed peptides were washed three times with DCM and **di****e**thyl**e**ther (**DEE**) each and dried. Cleavage was performed with cleavage solution (trifluoroacetic acid, 2.5 % water, 2.5 % triisopropylsilane, 2.5 % 2,2′-(Ethylendioxy)diethanthiol) for 1 h at room temperature under rigorous shaking. Peptides were precipitated in ice cold DEE, centrifuged and the supernatant collected, repeated three times in total.

N-terminal acetylation of the peptides was done on-resin. 2 mL DMF containing 90 µL of DIPEA and 50 µL of acetic anhydrite were added and allowed to react for 30 min at 25 °C under rigorous shaking. The resin was washed 3 times each with DMF, DCM and DEE and dried.

Preparative peptide purification was carried out using an GE ÄKTA pure (GE Healthcare, Chalfont St Giles, UK) system with a Luna 15 µm C18 100 Å column, 21.2 mm internal diameter, 250 mm length

(Phenomenex Inc., Torrance, CA), with eluant A (0.1% **t**rif**l**uoro**a**cetic acid (**TFA**) in water (v/v)) and eluent B (0.1% TFA in ACN (v/v)) with varying gradients starting from 10-50% (depending on the peptides solubility) to 100 % B in 60 minutes. UV absorption was read at λ = 214 nm 254 nm and 280 nm. The samples were frozen in liquid nitrogen, freeze-dried, and stored at -80 °C until further usage.

Denatonium-CH_2-NH_2 synthesis:

1.5 molar equivalents of lidocaine were mixed with 1 molar equivalent of tert-butyl-4-(bromomethyl)benzylcarbamate without solvents. The mixture was heated to 80 °C under stirring until the formation of a molten mixture and kept at 80 °C for 10 min to obtain a yellow solid. The solid was treated with a mixture of n-hexan and ethyl acetate (1:1) until the precipitation of white solid. The solid was filtrated and washed with a mixture of n-hexan and ethyl acetate (1:1). Afterwards, the solid was dried under vacuum. To remove the tert-butyloxycarbonyl protection-group, the solid was solubilized in pure TFA and shaken at 25 °C for 1 h. The resulting product was precipitated with the addition of DEE with a temperature of -20 °C. The precipitate was air-dried and then purified via reversed phase chromatography. Subsequently, the compound was characterized by HPLC and LC-MS.

Human sample collection and storage:

The study protocol was prepared in accordance with the Declaration of Helsinki and the criteria of good clinical practice. It was approved by the Ethics Committee of the University of Würzburg (file number 190/18). Written informed consent was obtained from all selected patients.

The study participants were recruited from patients visiting the department of Periodontology of the University Hospital of Würzburg, Germany between Oct/2019 to Dec/2020. Periodontally healthy patients as well as patients with periodontal diseases of varying severity (gingivitis, periodontitis) were included. The periodontally diseased patients were in different stages of treatment (untreated patients before active initial therapy, treated recall patients in regular supportive aftercare). Eligibility criteria were age 18 to 99 years. Patients of both sexes, with and without periodontal disease were included in the study.

Periodontal examinations were obtained from all study participants comprising the documentation of missing teeth, **p**robing **p**ocket **d**epths (**PPD**), recession, **b**leeding **o**n **p**robing (**BoP**), **c**linical **a**ttachment **l**evel (**CAL**), furcation involvement, tooth mobility and **pla**que **c**ontrol **r**ecord (**PlaCR**). Demographic details and general health profile of the participants including current intake of medications and frequency of smoking was documented using a questionnaire. CAL, PPD and BoP were recorded at 6 sites per tooth (mesio-buccal, buccal, disto-buccal, disto-oral, oral, mesio-oral) using a pressure calibrated computer aided Florida Probe handpiece. Any bleeding spot appearing within 30 seconds after probing was recorded as a BoP-positive site. Furcation involvement was determined using a manual furcation probe (Nabers Probe). The total amount of periodontal inflammation present in each patient was calculated from the recorded BoP, CAL and PPD scores using the PISA-Index [3]. Collected samples were quality-controlled hosted by the certified centralized biobank of the medical faculty of the University of Würzburg [4].

Total MMP-8 concentration determination:

Saliva samples were thawed at 4 °C, overnight, diluted 1:200 and analyzed by MMP-8 ELISA (Sigma-Aldrich, Taufkirchen, Germany) following provided protocols. In brief, samples and reagents were brought to room temperature. Samples were distributed on the sample plate, covered, and incubated for 2.5 h at room temperature under mild agitation. The sample plate was washed 4 times with wash buffer, supplied detection antibodies were added, allowed to bind 1 h at room temperature under mild agitation. Four wash steps followed, and finally streptavidin solution was added. The plate was covered and incubated for 45 min at room temperature under mild agitation, washed 4 additional times and detection substrate added, it was covered and incubated for 30 min at room temperature in the dark. The reaction was stopped, and absorbance measured at 450 nm in a microplate reader (Tecan Infinite F50, Crailsheim, Germany). Results were compared against a standard curve as supplied.

Supplementary Table 1. Overview of peptides and sensors detailed in this manuscript. Sequence Abbreviations: The one letter amino acid code was used, except for N-terminal acetylation (Ac), C-terminal functionalization with Den-CH_2-NH_2 (Den), L-azidohomoalanine (Aha) and for fluorescent dyes (DBCO-Sulfo-Cy5, AFDye488-DBCO, Sulfo-Cy-3-Alkin).

Abbreviation	Sequence	Characterization
S0	Ac-GPQGIAGA-Den	Supplementary Figure 4
S1	Ac-GSGS-GPQGIAGA-GSGS-Den	Supplementary Figure 2
S1c	IAGA-GSGS-Den	Supplementary Figure 2
S0p	Ac-GPQGIAGA	Supplementary Figure 4
S1p	Ac-GSGS-GPQGIAGA-GSGS	Supplementary Figure 4
X1	Ac-GPQGIWGA	Supplementary Figure 5
X2	Ac-GPQGYWGA	Supplementary Figure 5
X3	Ac-GPQAYWGA	Supplementary Figure 5
X4	Ac-GPLAYWGA	Supplementary Figure 5
Y1	Ac-GPLELVGA	Supplementary Figure 9
Y2	Ac-GPLEIVGA	Supplementary Figure 9
Y3	Ac-GPLALVGA	Supplementary Figure 9
Y4	Ac-GPLAIVGA	Supplementary Figure 9
Y5	Ac-GALELVGA	Supplementary Figure 10
Y6	Ac-GALEIVGA	Supplementary Figure 10
Y7	Ac-GALALVGA	Supplementary Figure 10
Y8	Ac-GALAIVGA	Supplementary Figure 10
Y3a	Ac-GSGS-GPLALVGA-GSGS-Aha	Supplementary Figure 11
Y5a	Ac-GSGS-GALELVGA-GSGS-Aha	Supplementary Figure 11
Y7a	Ac-GSGS-GALALVGA-GSGS-Aha	Supplementary Figure 11
SY3	Ac-GSGS-GPLALVGA-GSGS-Aha-DBCO-Sulfo-Cy5	Supplementary Figure 13
SY5	Ac-GSGS-GALELVGA-GSGS-Aha-AFDye488-DBCO	Supplementary Figure 13
SY7	Ac-GSGS-GALELVGA-GSGS-Aha-Sulfo-Cy-3-Alkin	Supplementary Figure 13
SX3	Ac-GSGS-GPQAYWGA-GSGS-Den	Supplementary Figure 12

Supplementary Table 2. Data overview from collected control saliva samples.

Patient Status	Patient number	ELISA [ng/mL]	Activity [ng/mL]	Cleavage SY3 [%]	Cleavage SY5 [%]	Cleavage SY7 [%]	Cleavage SX3 [%]
Control	**1**	23.57	55.46	5.40	4.88	6.37	1.40
Control	**2**	0.32	4.20	0.29	0.14	2.10	0.27
Control	**3**	36.73	31.90	11.05	11.04	6.33	1.89
Control	**4**	45.81	284.25	1.60	2.84	1.38	0.82
Control	**5**	10.38	5.85	1.07	0.17	0.89	0.33
Control	**6**	2.53	8.30	0.38	0.18	0.95	0.34
Control	**7**	0.60	2.34	1.39	0.16	1.81	0.30
Control	**8**	9.18	5.87	0.37	0.18	0.87	0.33
Control	**9**	13.21	20.29	0.31	0.15	0.12	0.29
Control	**10**	5.91	5.43	1.39	2.55	1.86	0.70
Control	**11**	300.59	29.02	3.77	4.69	3.42	1.17
Control	**12**	10.23	0.23	3.71	2.64	3.42	0.66
Control	**13**	2.99	6.28	2.96	2.11	2.50	0.69
Control	**14**	-	17.80	0.34	0.17	0.13	0.31
Control	**15**	56.04	73.70	4.60	3.56	2.58	1.95
Control	**16**	53.85	74.59	1.80	2.25	0.83	0.73

Supplementary Table 3. Data chart from collected periodontitis saliva samples.

Patient Status	Patient number	ELISA [ng/mL]	Activity [ng/mL]	Cleavage SY3 [%]	Cleavage SY5 [%]	Cleavage SY7 [%]	Cleavage SX3 [%]
Periodontitis	**17**	91.25	22.28	2.91	3.77	1.46	0.65
Periodontitis	**18**	30.17	42.06	5.20	6.20	4.24	2.04
Periodontitis	**19**	152.60	110.03	7.75	4.73	2.83	4.49
Periodontitis	**20**	111.46	97.00	6.76	4.23	3.01	2.93
Periodontitis	**21**	45.13	46.71	3.62	3.28	1.46	2.23
Periodontitis	**22**	25.15	33.92	7.68	5.69	6.38	1.94
Periodontitis	**23**	6.39	7.60	0.92	1.98	1.05	0.64
Periodontitis	**24**	20.75	24.16	2.58	2.43	1.32	1.20
Periodontitis	**25**	355.98	46.03	1.22	2.15	0.60	0.32
Periodontitis	**26**	59.03	67.21	6.74	4.20	4.80	2.11
Periodontitis	**27**	33.41	24.73	1.98	2.72	0.46	1.13
Periodontitis	**28**	34.70	31.87	2.81	3.80	1.24	1.52
Periodontitis	**29**	27.47	27.37	1.86	2.70	1.79	1.33
Periodontitis	**30**	72.92	53.17	3.46	4.51	2.36	0.32
Periodontitis	**31**	35.31	48.09	3.99	4.04	2.88	1.83
Periodontitis	**32**	39.16	71.76	4.92	3.91	2.67	2.58
Periodontitis	**33**	58.13	22.70	10.78	6.04	9.54	1.99
Periodontitis	**34**	831.11	195.81	8.98	8.39	7.48	0.78
Periodontitis	**35**	7.98	4.50	1.46	2.78	0.70	0.30
Periodontitis	**36**	40.47	52.47	4.66	3.78	4.09	1.48
Periodontitis	**37**	15.41	14.11	2.25	2.92	1.62	1.44
Periodontitis	**38**	19.07	17.72	2.22	2.84	1.93	1.09
Periodontitis	**39**	8.15	6.44	1.46	2.33	1.08	0.99
Periodontitis	**40**	18.65	8.30	2.71	3.09	1.59	1.10
Periodontitis	**41**	134.82	62.17	11.08	6.84	8.29	3.35
Periodontitis	**42**	119.64	39.76	4.06	3.83	3.71	1.24
Periodontitis	**43**	4.07	8.23	1.37	3.33	2.03	0.85
Periodontitis	**44**	527.08	335.12	14.82	6.70	9.13	3.17
Periodontitis	**45**	9.72	5.07	8.89	6.30	8.65	0.96
Periodontitis	**46**	76.21	52.19	14.96	6.31	12.48	2.12
Periodontitis	**47**	80.46	62.15	7.27	6.92	4.60	2.34
Periodontitis	**48**	45.83	42.79	5.46	4.73	5.00	1.69
Periodontitis	**49**	7.30	3.41	1.05	2.62	0.45	0.67
Periodontitis	**50**	173.23	63.31	3.42	3.65	1.98	1.64
Periodontitis	**51**	51.53	29.36	2.71	3.62	0.96	1.64
Periodontitis	**52**	76.50	36.09	6.67	5.28	4.11	2.41
Periodontitis	**53**	2.00	4.86	6.17	3.23	5.35	0.97
Periodontitis	**54**	82.19	55.41	2.78	3.55	2.96	1.24
Periodontitis	**55**	22.06	37.13	5.65	3.80	2.44	2.71
Periodontitis	**56**	932.89	129.81	3.84	4.46	2.59	0.76
Periodontitis	**57**	62.83	25.30	23.56	10.98	21.52	1.67
Periodontitis	**58**	7.22	1.51	0.79	2.35	0.70	0.33
Periodontitis	**59**	20.46	24.85	13.10	9.35	11.59	3.03
Periodontitis	**60**	26.40	23.26	1.03	2.21	1.17	0.32
Periodontitis	**61**	1282.65	437.77	25.35	18.84	10.19	7.92
Periodontitis	**62**	6.74	2.03	1.13	2.57	0.78	0.33
Periodontitis	**63**	5.24	21.62	0.97	2.60	0.37	0.64
Periodontitis	**64**	10.07	20.56	2.17	2.84	1.89	0.65
Periodontitis	**65**	24.22	32.93	2.34	2.44	0.65	1.12
Periodontitis	**66**	9.08	27.73	1.51	2.75	0.93	1.08

Supplementary Table 4. Correlations of active and total MMP-8 concentration with individual sensor cleavage.
Spearman's rank correlation coefficient displayed as rho and p-value as p (rho). P-values <0.05 were considered significant and displayed as * for values <0.05, ** for values <0.005 and *** for values <0.001.

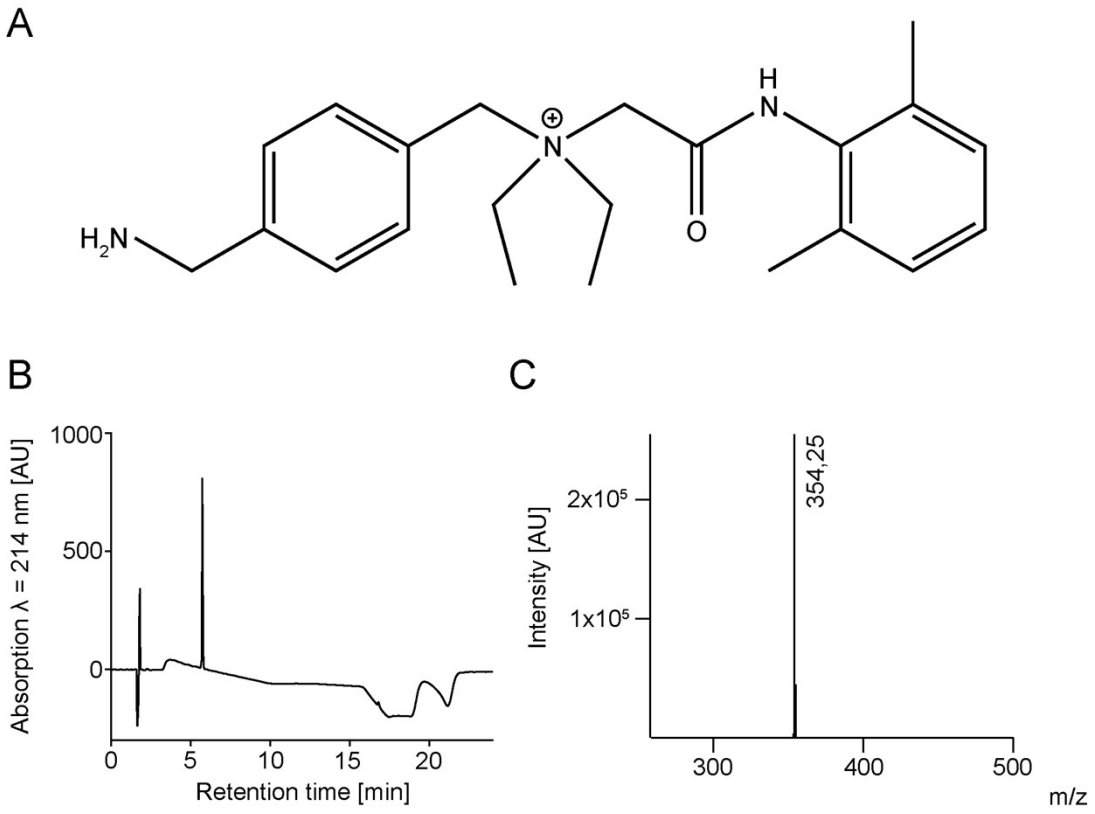
Input 1	Input 2	n	ρ/rho	p(ρ/rho)
Active MMP-8	SY3	65	0.5874	0.00000***
Active MMP-8	SY5	65	0.6007	0.00000***
Active MMP-8	SY7	65	0.4248	0.00038***
Active MMP-8	SX3	65	0.6403	0.00000***
Total MMP-8	SY3	66	0.6190	0.00000***
Total MMP-8	SY5	66	0.6475	0.00000***
Total MMP-8	SY7	66	0.4447	0.00021***
Total MMP-8	SX3	66	0.5612	0.00000***

Supplementary Table 5. Observed differences for different groups. Student's t-tests p-value displayed as p(t) and Mann-Whitney U tests p value displayed as p(U). P-values <0.05 were considered significant and displayed as * for values <0.05, ** for values <0.005 and *** for values <0.001.

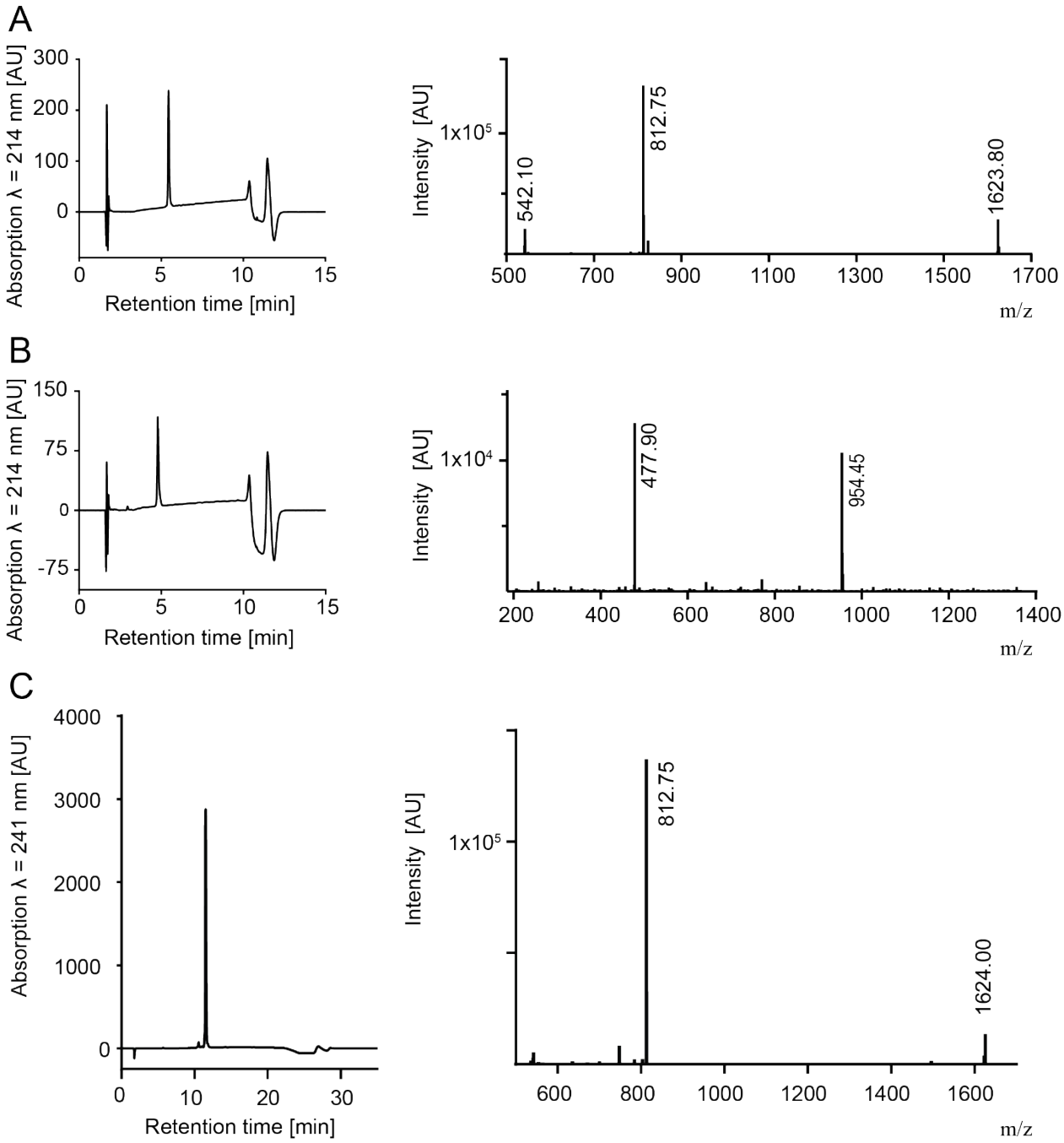
Parameter	Groups	n groups	p(t)	p(U)
Total MMP-8	Periodontitis vs. control	(50/15)	0.23	0.024 *
Active MMP-8	Periodontitis vs. control	(50/16)	0.52	0.087
SY3	Periodontitis vs. control	(50/16)	0.042 *	0.0072 **
SY5	Periodontitis vs. control	(50/16)	0.011 *	0.00061 ***
SY7	Periodontitis vs. control	(50/16)	0.13	0.17
SX3	Periodontitis vs. control	(50/16)	0.012 *	0.0015 **
Total MMP-8	Sex (m/f)	(18/31)	0.012*	0.14 *
Active MMP-8	Sex (m/f)	(18/31)	0.023 *	0.17 *

Supplementary Table 6. Observed correlations within periodontitis group for clinical parameters, MMP-8 concentration, and individual sensor cleavage. Kendall rank correlation coefficient displayed as tau and p-value as p(tau), Spearman's rank correlation coefficient displayed as rho and p-value as p (rho). P-values <0.05 were considered significant and displayed as * for values <0.05, ** for values <0.005 and *** for values <0.001.

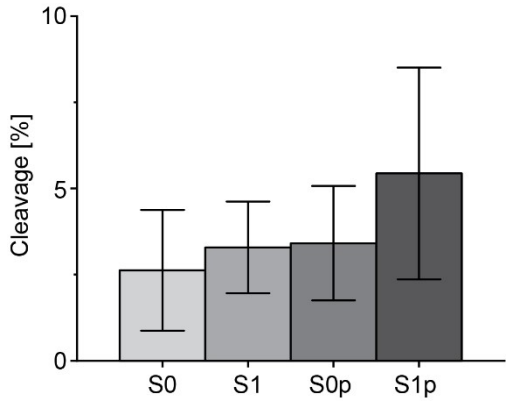
Input 1	Input 2	n	τ/tau	p(τ/tau)	ρ/rho	p(ρ/rho)
Total MMP-8	Age	49	0.2867	0.0037 **		
Active MMP-8	Age	49	0.2401	0.015 *		
SY3	Pocket depth	49	0.2237	0.023 *		
SY5	Pocket depth	49	0.2254	0.022 *		
SY7	Plaque	49			-0.2307	0.019 *
SY7	Pocket depth	49	0.1914	0.052		
SX3	Pocket depth	49	0.2152	0.029 *		



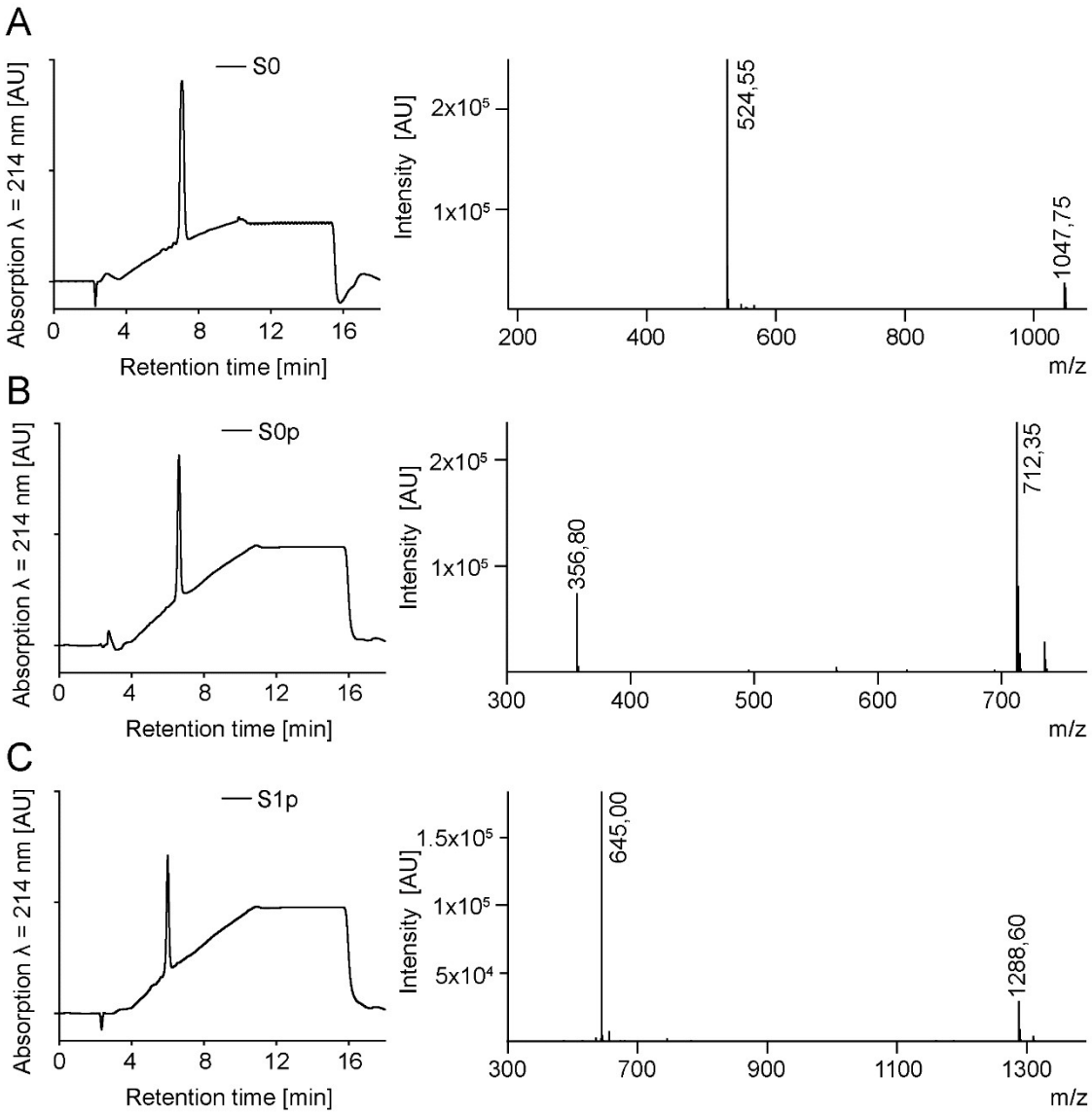
Supplementary Figure 1. Characterization of denatonium derivative Den-CH_2-NH_2. (A) Structure of Den-CH_2-NH_2. (B) HPLC chromatogram of Den-CH_2-NH_2 indicating a purity of >95 %. (B) LC-MS analysis of Den-CH_2-NH_2 showing a m/z of 354.25 for a calculated m/z of 354.25.



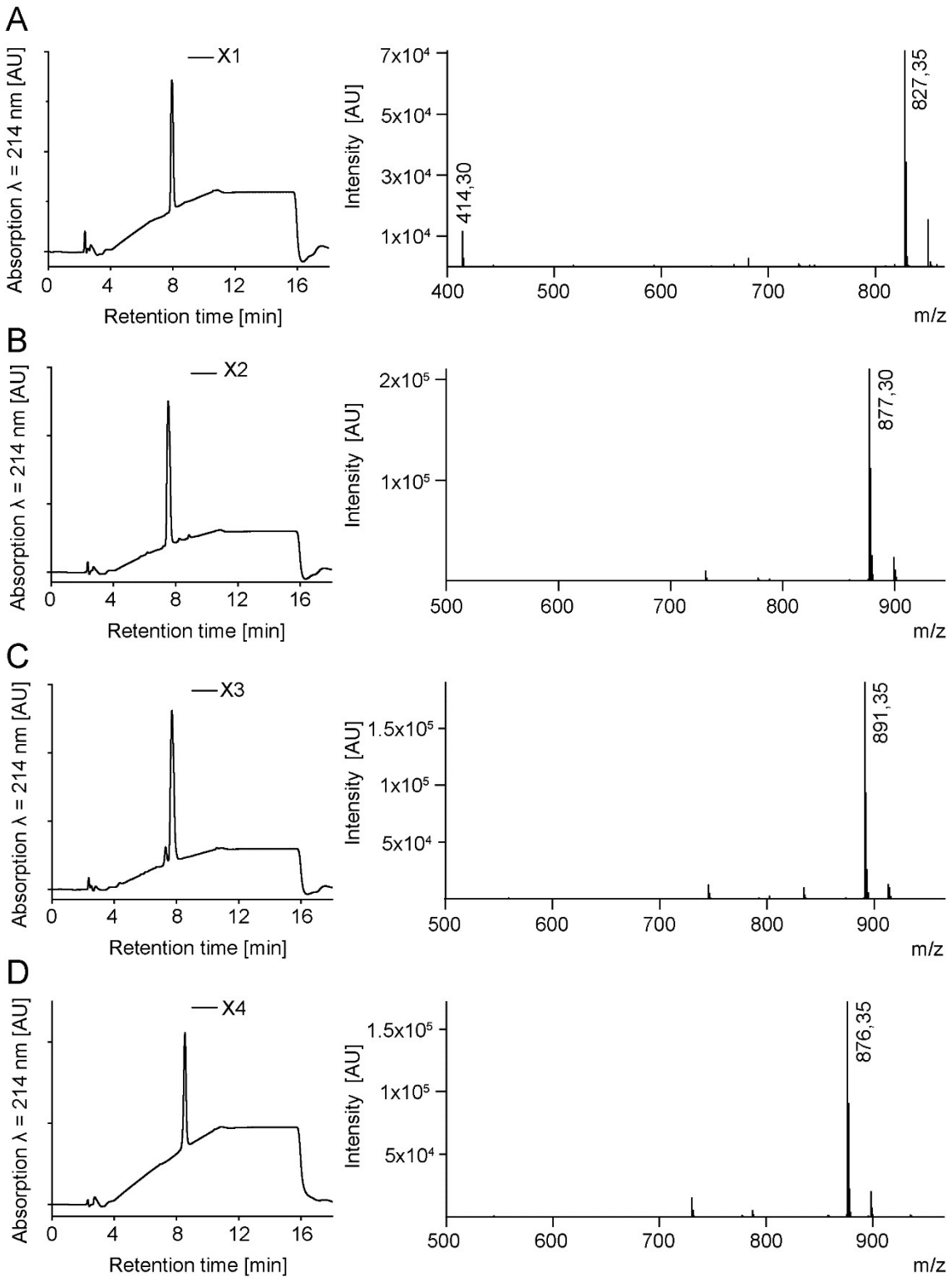
Supplementary Figure 2. Characterization of S1 and S1c. (A) HPLC chromatogram of S1 indicating a purity of >95 %. LC-MS analysis of S1 indicating an observed m/z of 1623.80 for a calculated m/z of 1624.13. (B) HPLC chromatogram of S1c indicating a purity of >95 %. LC-MS analysis of S1c indicating an observed m/z of 954.45 for a calculated m/z of 954.54. (C) HPLC chromatogram of long-term storage (> 2.5 years) of S1 indicating a purity of >95 %. LC-MS analysis of S1 indicating an observed m/z of 1624.00 for a calculated m/z of 1624.13.



Supplementary Figure 3. Cleavage for different sensor stages. (A) Cleavage results for S0, S1, S0p and S1p with 17.5 nM MMP-8 for 15 min at 37 °C. Experiments were performed with n = 3.

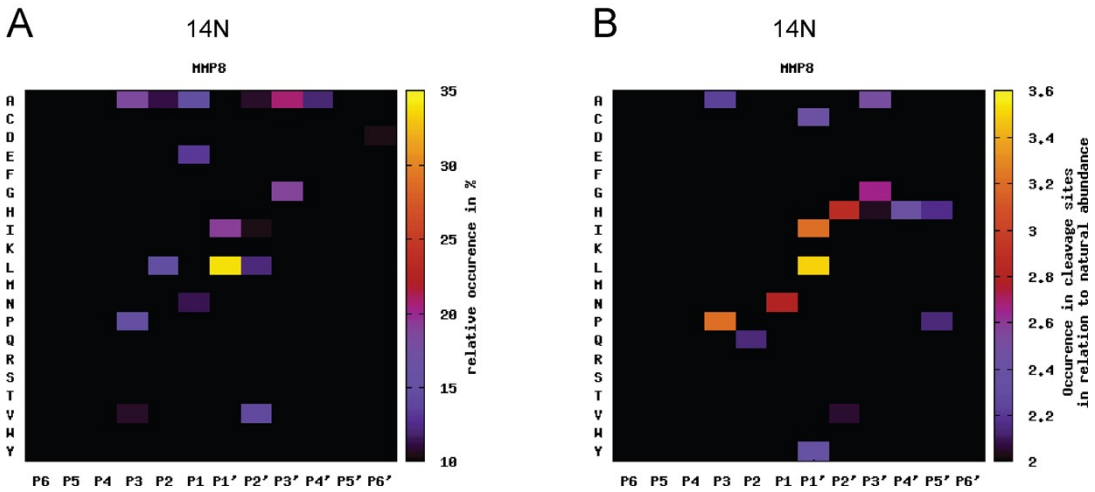


Supplementary Figure 4. Characterization of S0, S1p and S0p. (A) LC-MS analysis of S0 indicating a purity of >95% and an observed m/z of 1047.75 for a calculated m/z of 1047.60. (B) LC-MS analysis of S0p indicating a purity of >95% and an observed m/z of 712.35 for a calculated m/z of 712.34. (C) LC-MS analysis of S1p indicating a purity of >95% and an observed m/z of 1288.60 for a calculated m/z of 1288.56.

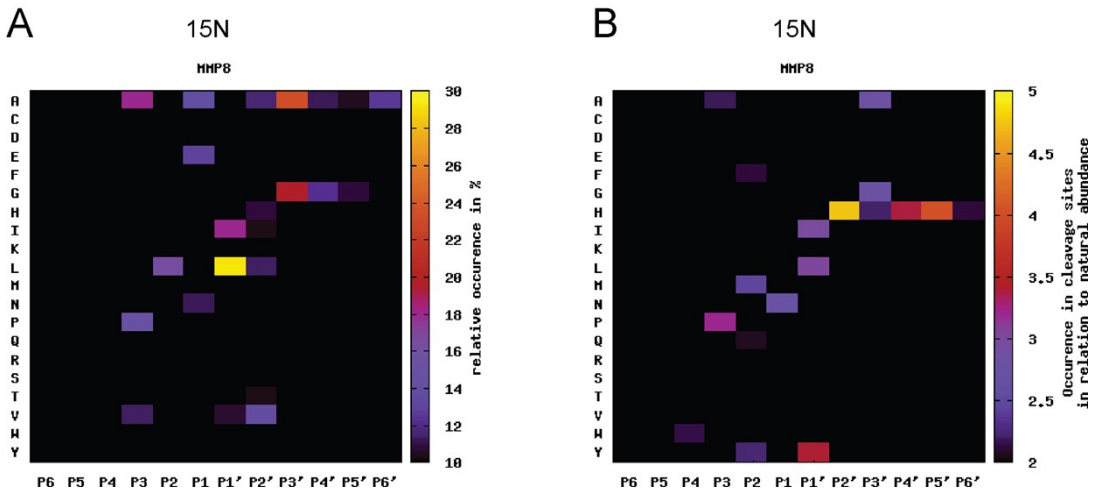


Supplementary Figure 5. Characterization of X1-4. (A) LC-MS analysis of X1 indicating a purity of >95% and an observed m/z of 827.35 for a calculated m/z of 827.32. (B) LC-MS analysis of X2 indicating a purity of >95% and an observed m/z of 877.30 for

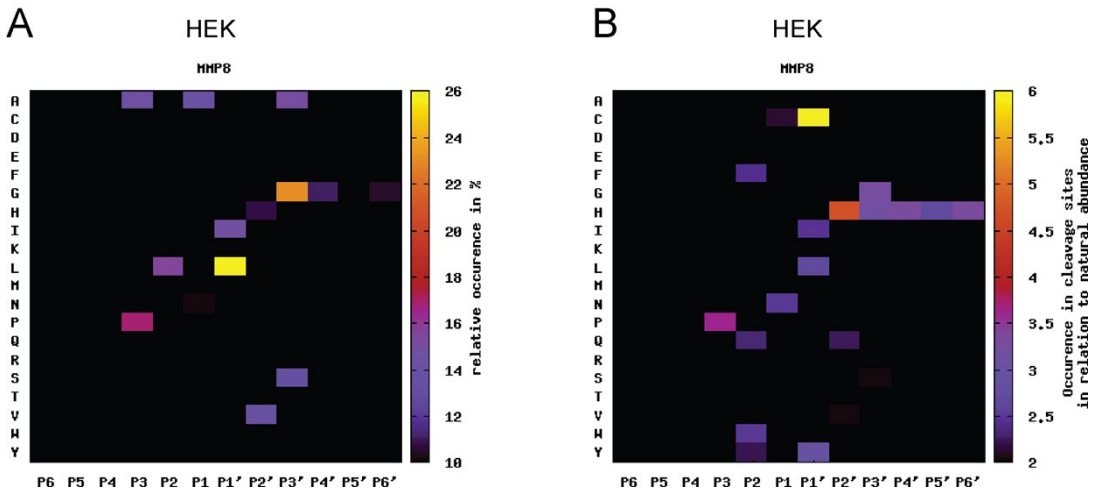
a calculated m/z of 877.37. (C) LC-MS analysis of X3 indicating a purity of >90% and an observed m/z of 891.35 for a calculated m/z of 891.38. (D) LC-MS analysis of X4 indicating a purity of >95% and an observed m/z of 876.35 for a calculated m/z of 876.41.



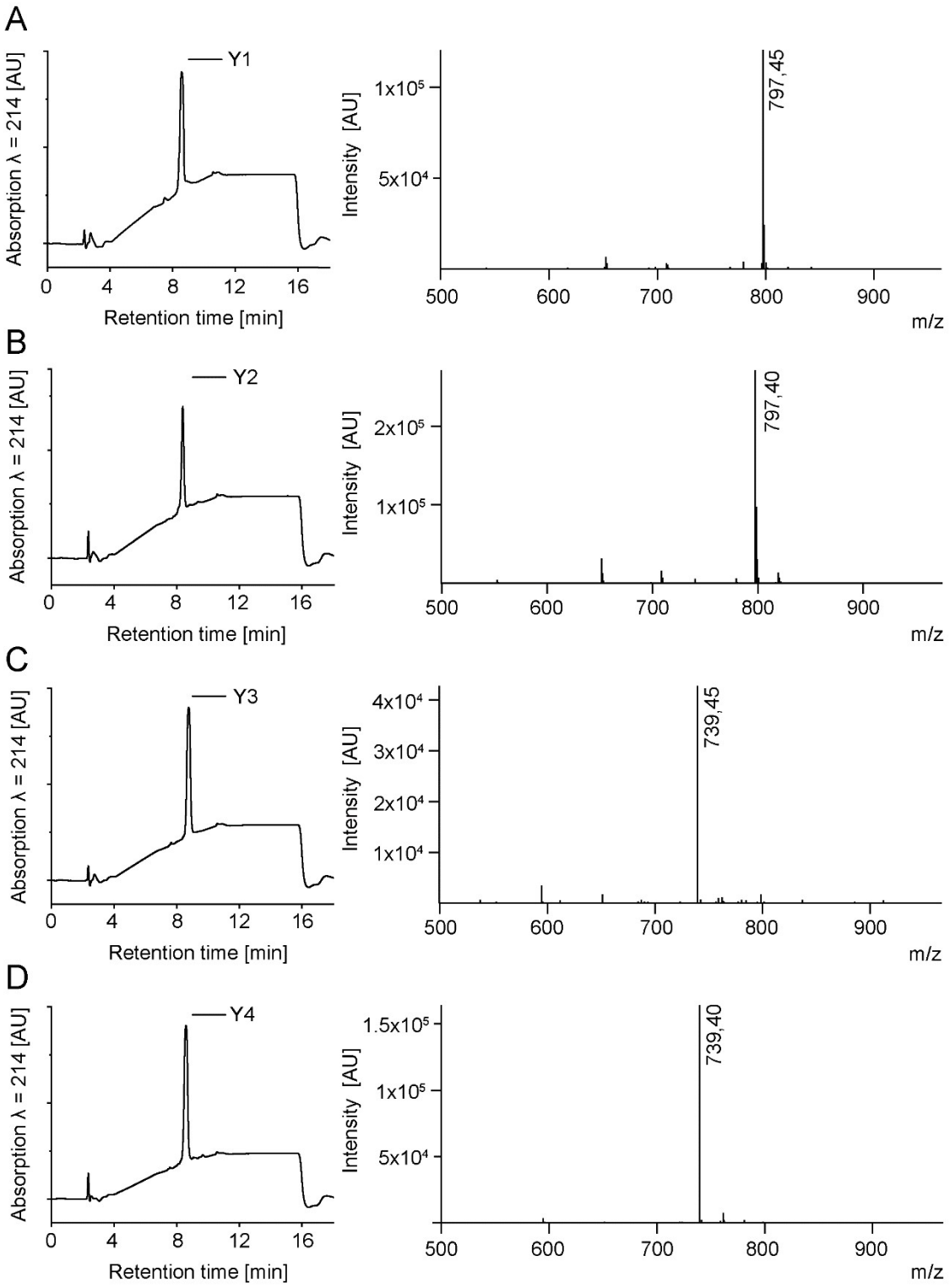
Supplementary Figure 6. MMP-8 PICS results acquired with 14N *E. coli* proteome. (A) relative occurrence in %. (B) Occurrence in cleavage sites in relation to natural abundance.



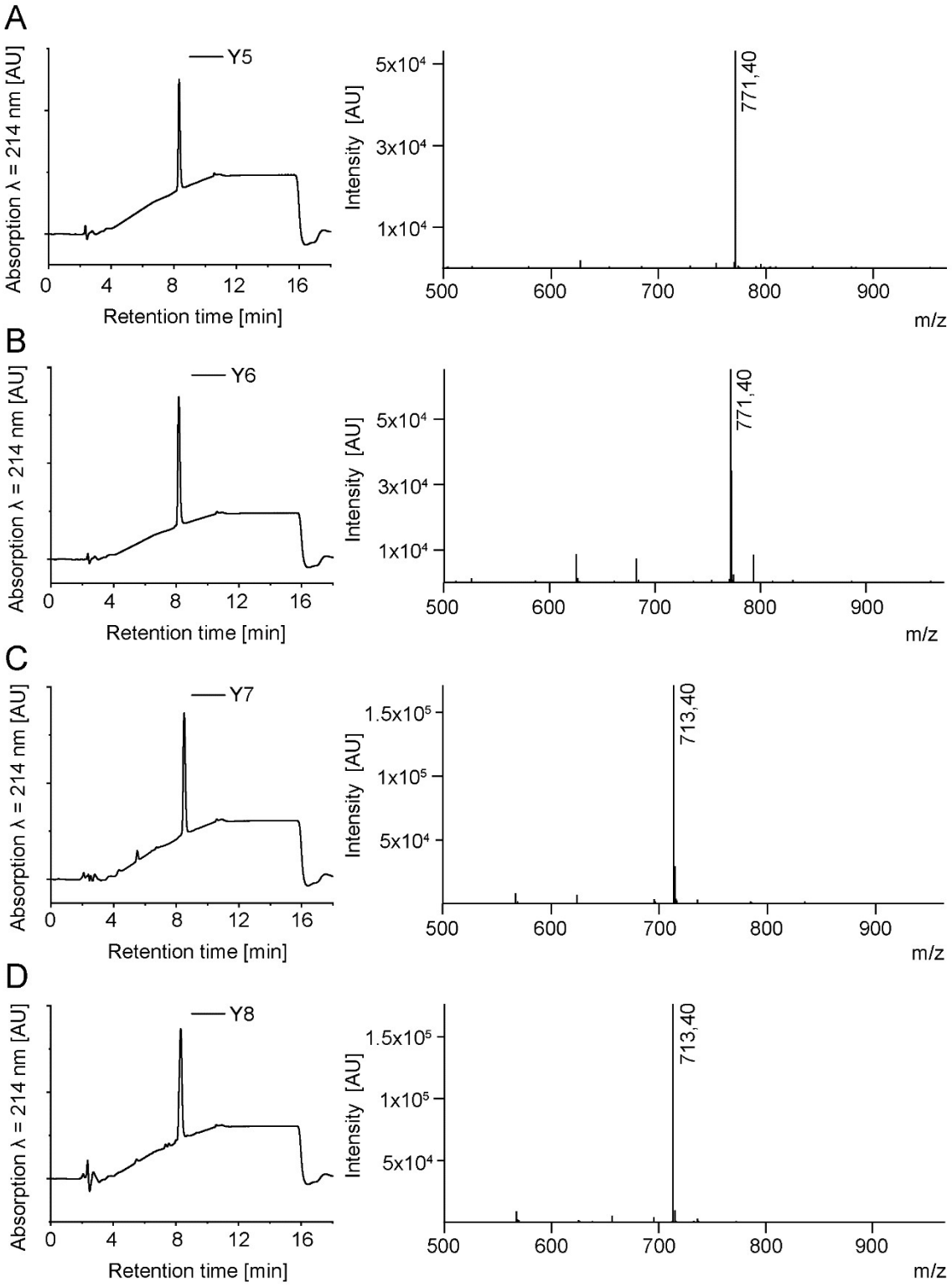
Supplementary Figure 7. MMP-8 PICS results acquired with 15N *E. coli* proteome. (A) relative occurrence in %. (B) Occurrence in cleavage sites in relation to natural abundance.



Supplementary Figure 8. MMP-8 PICS results acquired with HEK proteome. (A) Relative occurrence in %. (B) Occurrence in cleavage sites in relation to natural abundance.

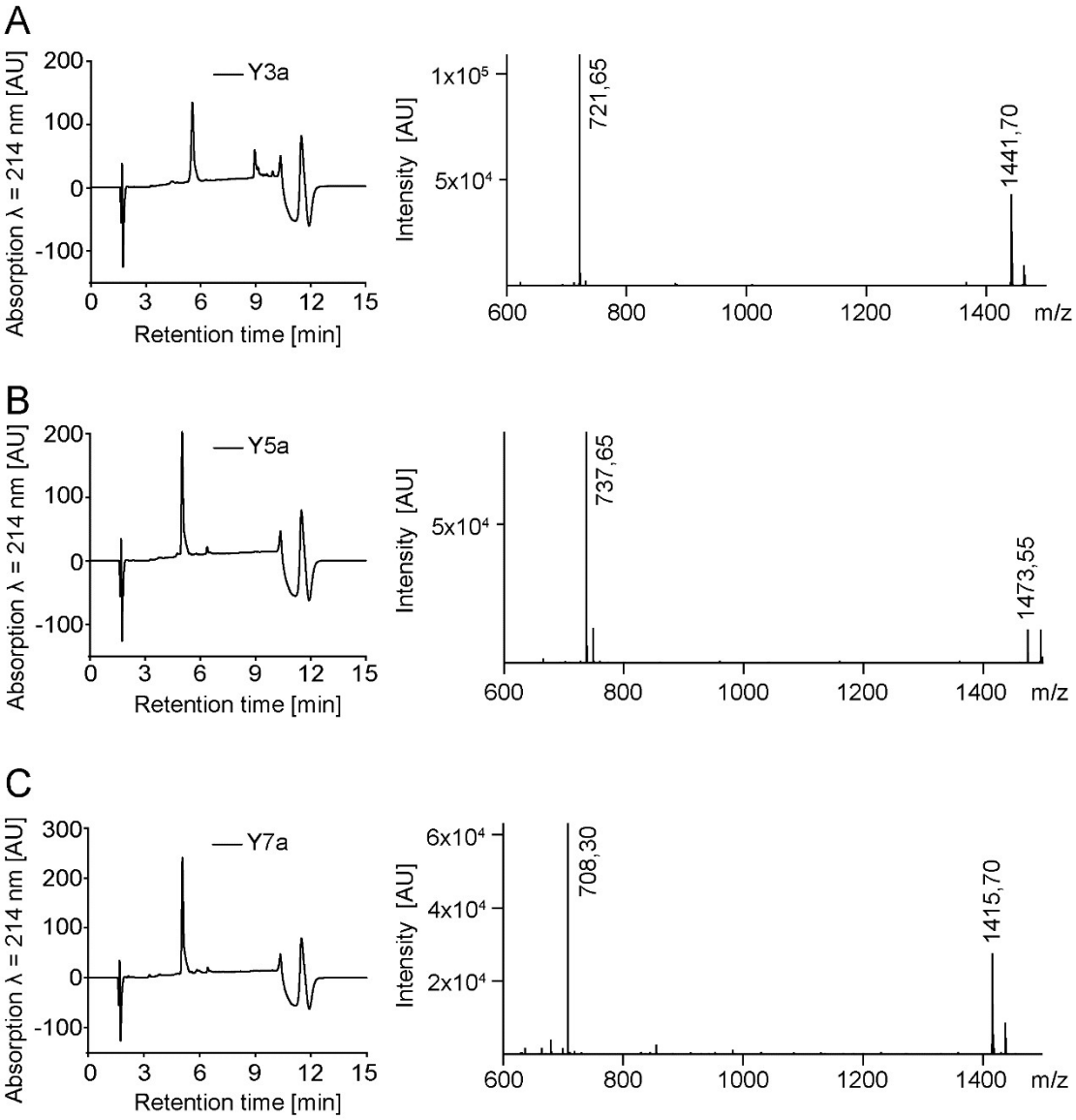


Supplementary Figure 9. Characterization of Y1-4. (A) LC-MS analysis of Y1 indicating a purity of >95% and an observed m/z of 797.45 for a calculated m/z of 797.42. (B) LC-MS analysis of Y2 indicating a purity of >95% and an observed m/z of 797.40 for a calculated m/z of 797.42. (C) LC-MS analysis of Y3 indicating a purity of >90% and an observed m/z of 739.45 for a calculated m/z of 739.42. (D) LC-MS analysis of Y4 indicating a purity of >95% and an observed m/z of 739.40 for a calculated m/z of 739.42.

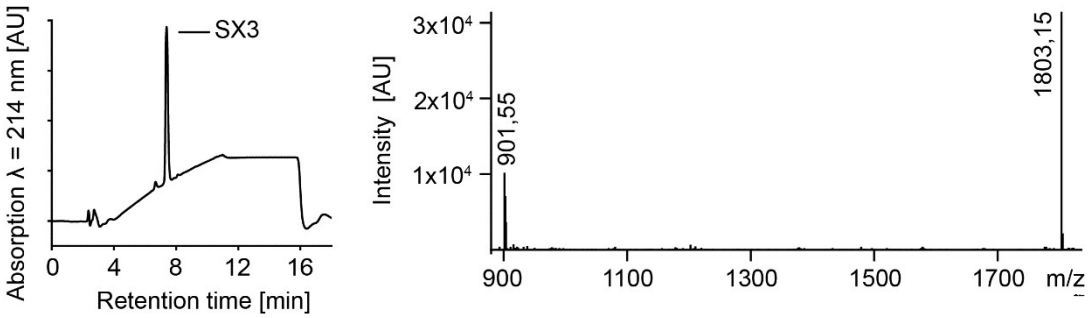


Supplementary Figure 10. Characterization of Y5-8. (A) LC-MS analysis of Y5 indicating a purity of >95% and an observed m/z of 771.40 for a calculated m/z of 771.41. (B) LC-MS analysis of Y6 indicating a purity of >95% and an observed m/z of 771.40

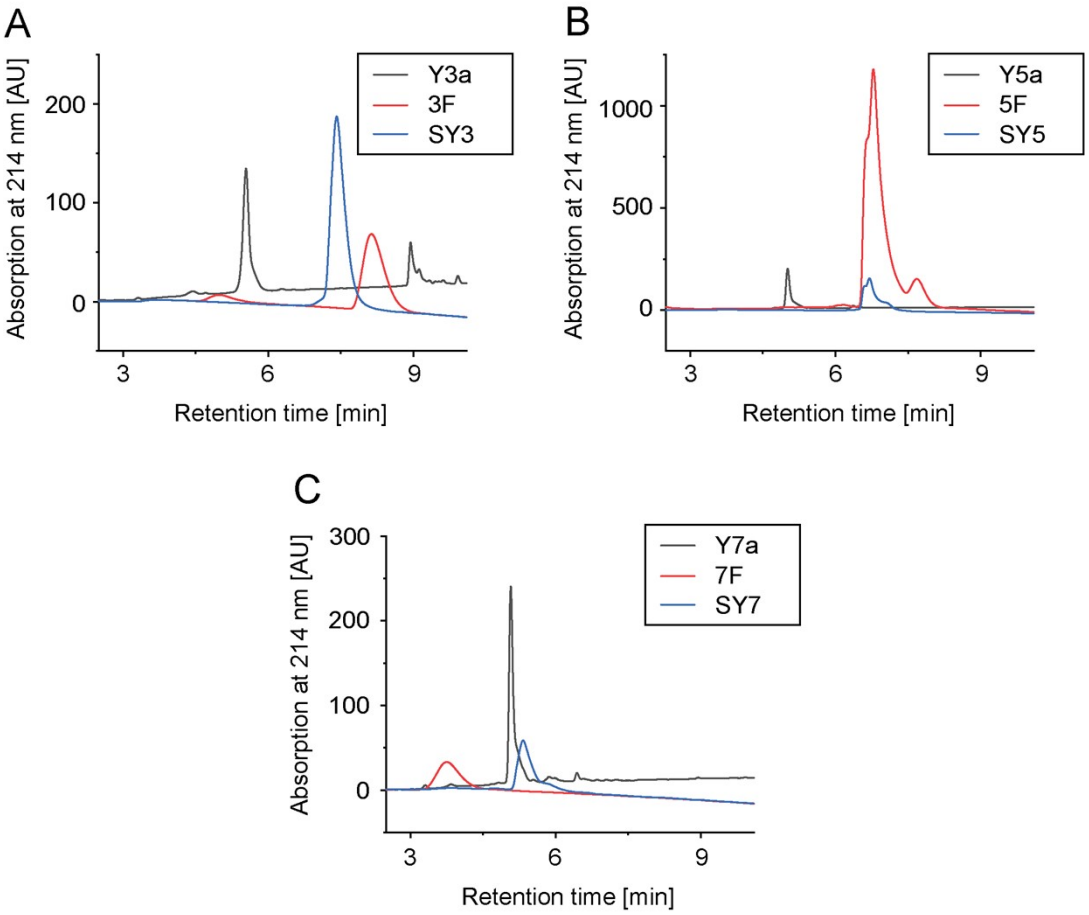
for a calculated m/z of 771.41. (C) LC-MS analysis of Y7 indicating a purity of >90% and an observed m/z of 713.40 for a calculated m/z of 713.40. (D) LC-MS analysis of Y8 indicating a purity of >95% and an observed m/z of 713.40 for a calculated m/z of 731.40.



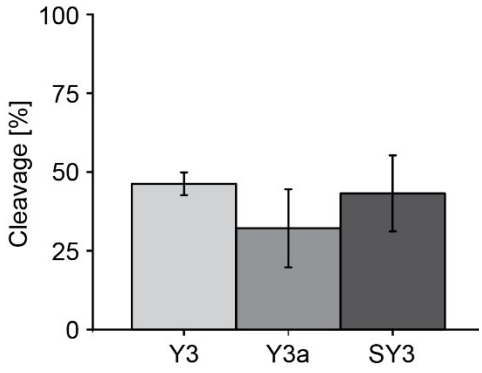
Supplementary Figure 11. Characterization of Y3a, Y5a and Y7a. (A) HPLC chromatogram of Y3a indicating a purity of >60 %. LC-MS analysis of Y3a indicating an observed m/z of 1441.70 for a calculated m/z of 1441.71. (B) HPLC chromatogram of SY5 indicating a purity of >95 %. LC-MS analysis of SY5 indicating an observed m/z of 1473.55 for a calculated m/z of 1473.70. (C) HPLC chromatogram of SY7 indicating a purity of >95 %. LC-MS analysis of SY7 indicating an observed m/z of 1415.70 for a calculated m/z of 1415.69.



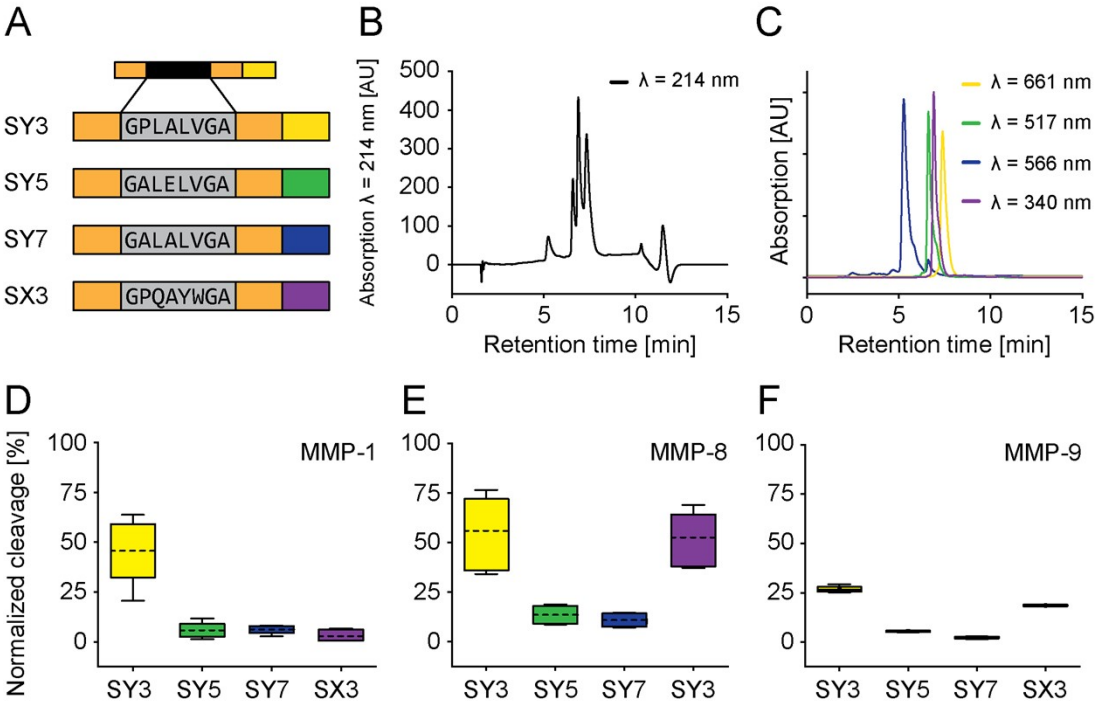
Supplementary Figure 12. Characterization of SX3. (A) LC-MS analysis of SX3 indicating a purity of >90% and an observed m/z of 1803.15 for a calculated m/z of 1802.85.



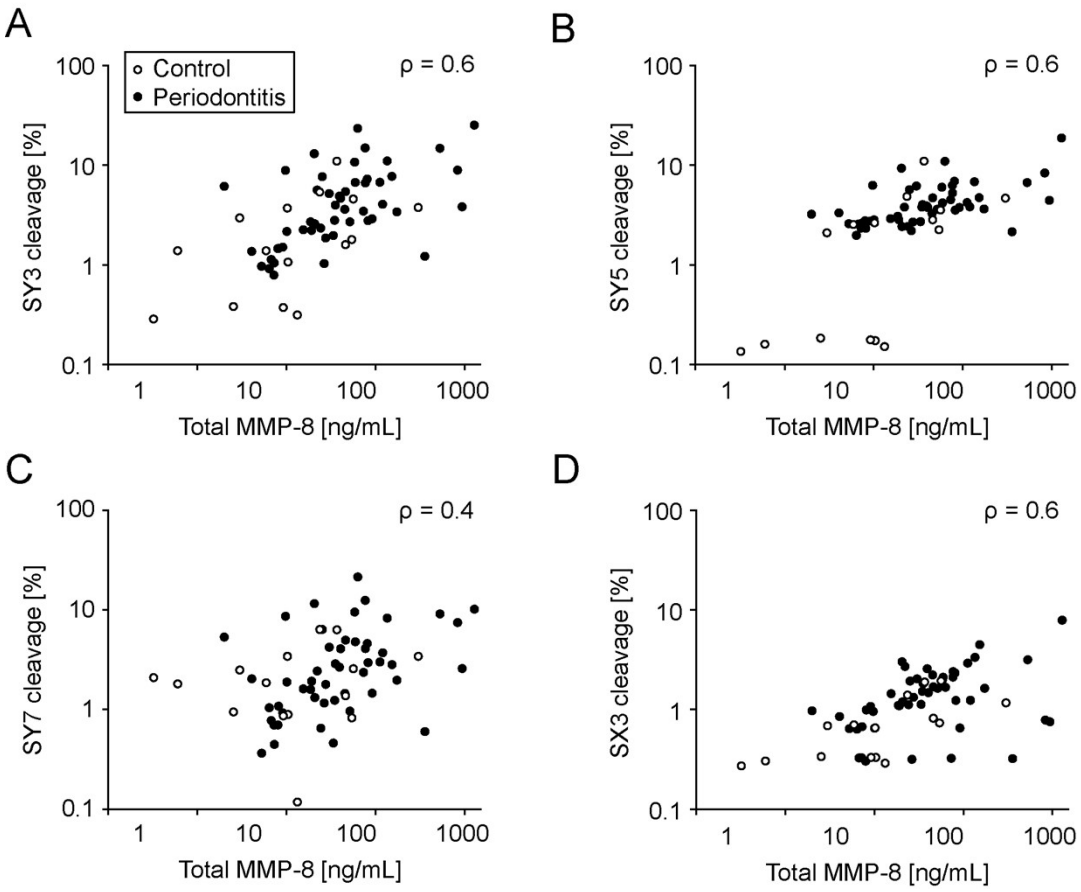
Supplementary Figure 13. HPLC characterization of SY3, SY5 and SY7. (A) HPLC chromatogram of Y3a (grey), 3F (DBCO-Sulfo-Cy5, red) and SY3 (blue). (B) HPLC chromatogram of Y5a (grey), 5F (AFDye488-DBCO, red) and SY5 (blue). (C) HPLC chromatogram of Y7a (grey), 7F (Sulfo-Cy-3-Alkin, red) and SY7 (blue).



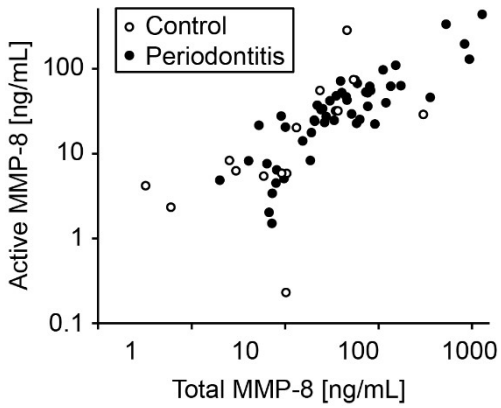
Supplementary Figure 14. Cleavage for different sensor stages. (A) Cleavage results for Y3, Y3a, and SY3 with 17.5 nM MMP-8 for 15 min at 37 °C. Experiments were performed with n = 3.



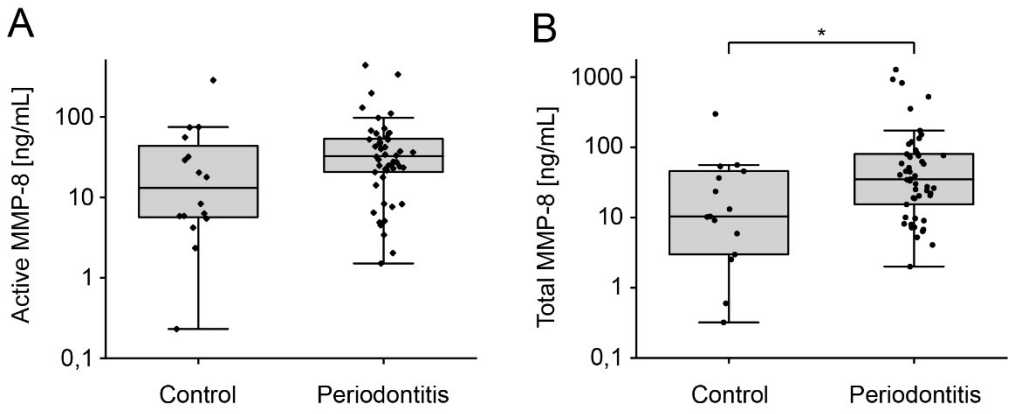
Supplementary Figure 15. Characterization of multiplex capable fluorescent sensors in mixture. (A) Overview sensors. (B) HPLC chromatogram acquired at λ = 214 nm with SY3, SY5, SY7 and SX3 in mixture. (C) HPLC chromatogram acquired at individual wavelengths for SY3, SY5, SY7 and SX3 in mixture. (D) Normalized MMP-1 cleavage for SY3, SY5, SY7 and SX3 in mixture. (E) Normalized MMP-8 cleavage for SY3, SY5, SY7 and SX3 in mixture. (F) Normalized MMP-9 cleavage for SY3, SY5, SY7 and SX3 in mixture. Box plots show the mean as dotted line, whiskers indicate outliers and the range of the box range indicates 25% and 75% percentiles, respectively.



Supplementary Figure 16. Cleavage of individual sensors in human saliva shown with corresponding total MMP-8 concentration determined via ELISA. Data presented in double logarithmic scale, with n indicating the amount of presented data points. (A) SY3 cleavage shown with corresponding total MMP-8 concentration. (B) SY5 cleavage shown with corresponding total MMP-8 concentration. (C) SY7 cleavage shown with corresponding total MMP-8 concentration. (D) SX3 cleavage shown with corresponding total MMP-8 concentration.



Supplementary Figure 17. Active MMP-8 concentration against total MMP-8 concentration. Active MMP-8 was determined via MMP-8 activity assay and total MMP-8 was determined via ELISA. Data presented in double logarithmic scale.



Supplementary Figure 18. MMP-8 concentrations for healthy control and periodontitis group. Data presented in logarithmic scale with box plots showing the median as solid line, whiskers as outliers, and the range of the box range as 25% and 75% percentiles, respectively. (A) Active MMP-8 concentration for healthy control and periodontitis group. (B) Total MMP-8 concentration for healthy control and periodontitis group. P-values <0.05 were considered significant and displayed as * for values <0.05, ** for values <0.005 and *** for values <0.001.

List of abbreviations:

- BOP Bleeding on probing
- MMP Matrix-metalloproteinase
- OTF Oral-thin-film
- PICS Proteomic identification of protease cleavage sites
- Den denatonium
- AP Aminopeptidases
- HPLC High performance liquid chromatography
- HeK human embryonic kidney
- PBS phosphate buffered saline
- Tris tris(hydroxymethyl)aminomethane
- SDS sodium dodecyl sulfate
- DTT dithiothreitol
- PMSF phenylmethylsulfonyl fluoride
- BCA bicinchoninic acid assay
- IAA iodoacetamide
- HEPES 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
- TCA trichloracetic acid

- FA formic acid
- ACN acetonitrile
- LC liquid chromatography
- MS mass spectrometry
- Fmoc fluorenylmethoxycarbonyl protecting group
- CTC 2-Chlorotrityl chloride
- DCM dichlormethane
- DIPEA diisopropylethylamine
- DIC N,N′-Diisopropylcarbodiimide
- DEE diethylether
- TFA trifluoroacetic acid
- APMA 4-Aminophenylmercuric acetate
- AUC area under the curve
- EDTA ethylenediaminetetraacetic acid
- PPD Probing pocket depths
- BOP Bleeding on Probing
- CAL clinical attachment level
- PLaCR plaque control record

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[4] J. Geiger, Both, S., Kircher, S., Neumann, M., Rosenwald, A. and Jahns, R., Hospital-integrated Biobanking as a Service – The Interdisciplinary Bank of Biomaterials and Data Wuerzburg (ibdw). Open Journal of Bioresources, 5, p.6. (2018).