Supplementary Material (ESI) for Chemical Communications

Supporting Information

Surface Immobilization of Biomolecules by Click Sulfonamide Reaction

Thimmaiah Govindaraju,[ac] Pascal Jonkheijm,[ac] Lars Gogolin,[b] Hendrik Schroeder,[d] Christian F. W. Becker,[be] Christof M. Niemeyer,[d] and Herbert Waldmann[ac]*

[a]*Department of Chemical Biology,*
[b]*Department of Physical Biochemistry,*
Max-Planck-Institut für Molekulare Physiologie,
Otto-Hahn-Strasse 11,
44227 Dortmund (Germany),
Fax: (+49) 231-133-2499.
E-mail: herbert.waldmann@mpi-dortmund.mpg.de
[c]*Chemische Biologie,*
[d]*Biologisch-Chemische Mikrostrukturtechnik,*
Fachbereich Chemie, Technische Universität Dortmund,
44227 Dortmund (Germany).
[e]*Technische Universität München, Fakultät Chemie,*
85747 Garching (Germany) and
Center of Integrated protein science Munich (CIPSM).

General: All chemicals were purchased from Sigma-Aldrich in the highest purity available unless otherwise mentioned and used without further purification. TBTA was synthesized from tripropargyl amine (GFS Chemicals, Inc.) and benzyl azide (Alfa Aeser).[1] Anti-phosphotyrosine antibody-biotin-streptavidin-Cy5-conjugate (50nM) was prepared in biotin-TETBS (TETBS containing 800 μM biotin to block free binding sites of streptavidin). Other reagents were obtained as follows anti-phosphotyrosine antibody-biotin from Leico technologies Inc. Streptavidin-Cy5 from Anova. Alexa647-labeled Concanavalin A from Molecular Probes and PTP1B from BIOMOL Research Laboratories.

Slide functionalization: Dry DMF (60 mL), diisopropylethylamine (1.2 mM), amine deprotected tauryl-sulfonyl azide **7**[2] (0.6 mM)), and diisopropylcarbodiimide ((0.5 mM) were added to a Schlenk flask containing a COOH-terminated PAMAM glass slides (Chimera Biotec, Dortmund) mounted on a teflon rack. The mixture was stirred overnight at room temperature under argon. The supernatant solution was removed, and the slides were stirred in dry CH_2Cl_2 for 30 min. After thoroughly rinsing the slide with CH_2Cl_2, the slides were dried in vaccuo. The slides were stored under argon until further use.

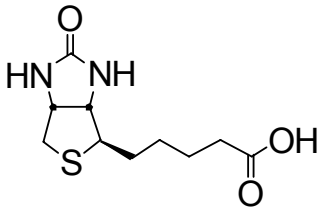
Spotting: The spotting process was carried out using a GeSiM Nanoplotter (Gesim, Dresden, Germany) by spotting 250 pL droplets of sample solutions (in H_2O:DMF or PBS buffer; spot size 400 μm diameter) followed by incubation in a humidity chamber (50%) containing saturated solution of potassium carbonate for 6 h at room temperature.

Preparation of spotting solutions of small molecules and peptides and detection of immobilization: 1 mM solutions of alkyne in water:DMF (2:1), 1 mM solutions of TBTA, $Cu(CH_3CN)_4PF_6$, sodium ascorbate and 10 mM solution of $NaHCO_3$ were prepared separately. To a 80 μL of alkyne solution (1 mM) in a well plate, 2 μL of $Cu(CH_3CN)_4PF_6$ (2 mol%), 2 μL of TBTA (2 mol%), 4 μL of sodium ascorbate (4 mol%), and 10 μL of $NaHCO_3$ (1 eq.) were added before spotting the solution onto a sulfonyl azide functionalized glass slide. Additional spotting solutions of alkynes (0.1, 0.01 mM for biotin-alkyne **1** and 0.1, 0.01, 0.001 mM for phosphopeptides) were prepared from the corresponding diluted stock solutions of the alkynes and reagents. Similarly spotting solutions of

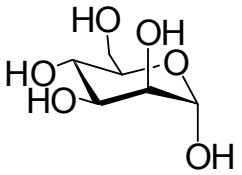
mannose-alkyne **2** with concentrations 10, 1, 0.1, 0.01 and 0.001 mM were prepared. Spotting solutions of negative controls were prepared from the compounds without alkyne functionality.

The slides were washed thoroughly with DMF, water and buffer (TETBS, pH 7.5 plus Tween 20, 0.5%). To reduce nonspecific binding of reagents, all slides were incubated for 30 min with a blocking solution containing milk powder (Chimera Biotec, Dortmund) after small-molecule or phosphopeptide immobilization. After drying, the hybridization chambers (Schleicher & Schuell) were tied on the slide and for the experiments shown, dried by centrifugation and the fluorescence intensity was measured using a microarray laser scanning system (Axon; pmt: 500; 100% laser power). Signals were analyzed and quantified using GenePix pro 4.1 software (Axon). For the experiments shown in Figure 3A, the binding of anti-phosphotyrosine antibody-Biotin-Strep-Cy5 conjugate (100 nm) was carried; b) for the lectin binding experiment (Figure 2B), the mannose arrays slides were blocked with HEPES buffer (10 mM; pH 7.5; 1mM $CaCl_2$, 1mM $MnCl_2$, 100 mM NaCl, 1% BSA, w/v) and then incubated with Alexa-647 labeled concanavalin A (100 nM) in the same buffer for 30 min at room temperature.

Click sulfonamide reaction on surface (for proteins): A solution of Cherry-Ypt7-alkyne **11** (100 μM) in PBS buffer (pH 7.5), 100 μM solutions of TBTA, $Cu(CH_3CN)_4PF_6$, sodium ascorbate, and 1 mM solution of $NaHCO_3$ were prepared separately. To a 80 μL of Cherry-Ypt7-alkyne solution (100 μM) in a well plate, 2 μL of $Cu(CH_3CN)_4PF_6$ (2 mol%), 2 μL of TBTA (2 mol%), 4 μL of sodium ascorbate (4 mol%), and 10 μL of $NaHCO_3$ (1 eq.) were added just before spotting the solution onto a sulfonyl azide functionalized glass slide. Similarly, three additional spotting solutions of Cherry-Ypt7-alkyne **11** (50, 25 and 12.5 μM) were prepared from the corresponding diluted stock solutions of the protein and reagents. Spotting solutions of negative controls were prepared from the compounds without alkyne functionality. Spotting solutions of Cherry-Ypt7-alkyne **11** in PBS buffer (pH 7.5) were spotted onto sulfonyl azide functionalized dendrimer glass slides and the slides were incubated in a humidity chamber for 6 h. The slides were then washed with PBST buffer (PBS buffer plus Tween 20 0.5%) and water and dried by centrifugation. The slides were then scanned for fluorescence.



Biotin (NC for biotin-alkyne **1**)



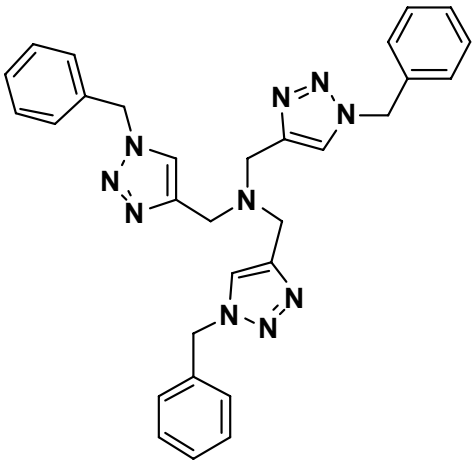
α-D-Mannose (NC for-
α-D-mannose-alkyne **2**)

Pro-Asp-Val-Leu-Glu-Tyr-pTyr-Lys-Asn-Glu-His-Ala-Lys

Phosphopeptide **3a** (NC for phosphopeptide **3**)

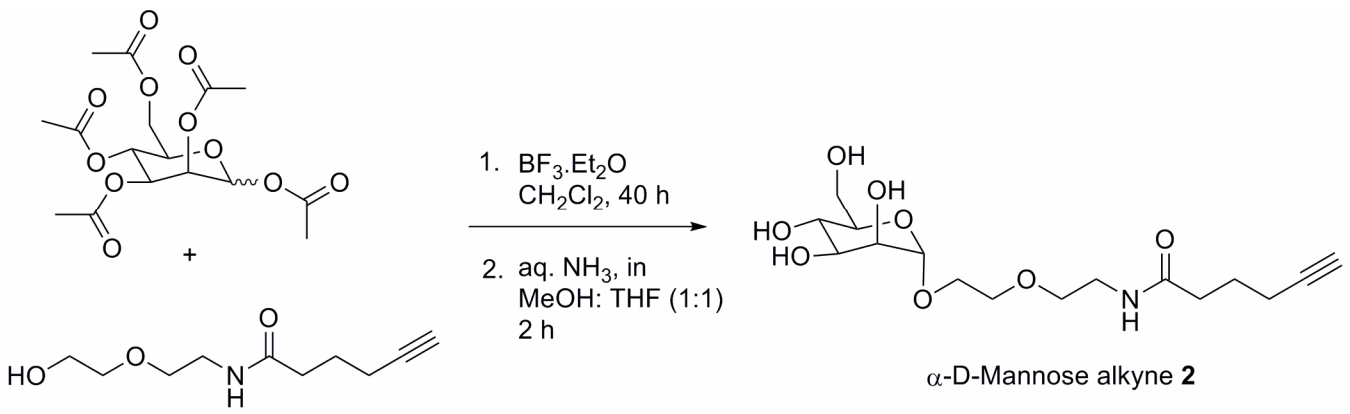
Arg-Asp-Ile-Asn-Ser-Leu-pTyr-Glu-Val-Ser-Arg-Met-Tyr

Phosphopeptide **4a** (NC for phosphopeptide **4**)



TBTA

Fig. **S1** Structures of negative controls used for spotting along with biotin-alkyne **1**, α-D-mannose-alkyne **2** and alkyne-modified phosphopeptides **3** and **4**, and the TBTA ligand used in click sulfonamide reaction.



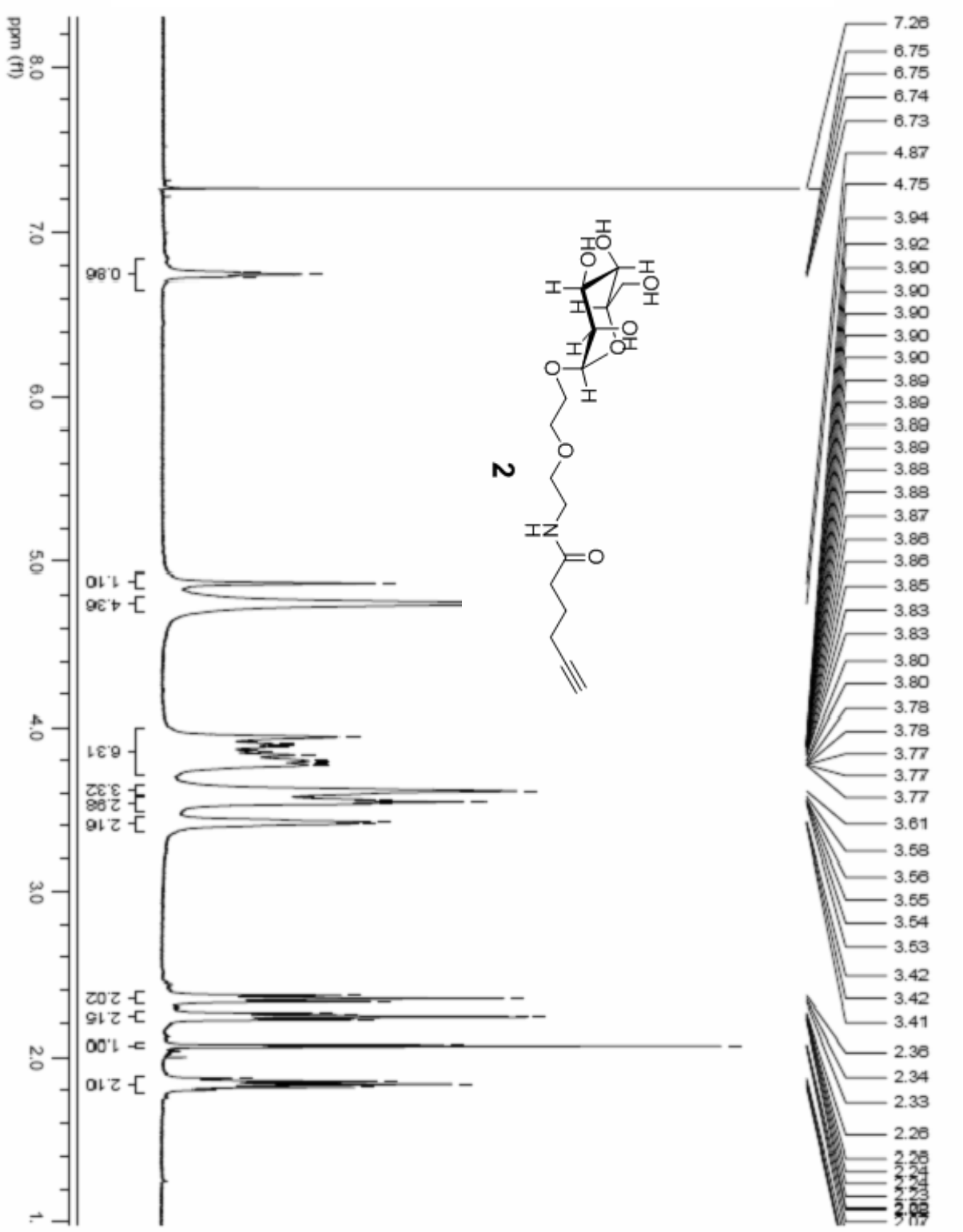
Scheme S1. Preparation of mannose with alkyne linker from α–D-mannose for the use in the immobilization onto sulfonylazide surface via click sulfonamide reaction

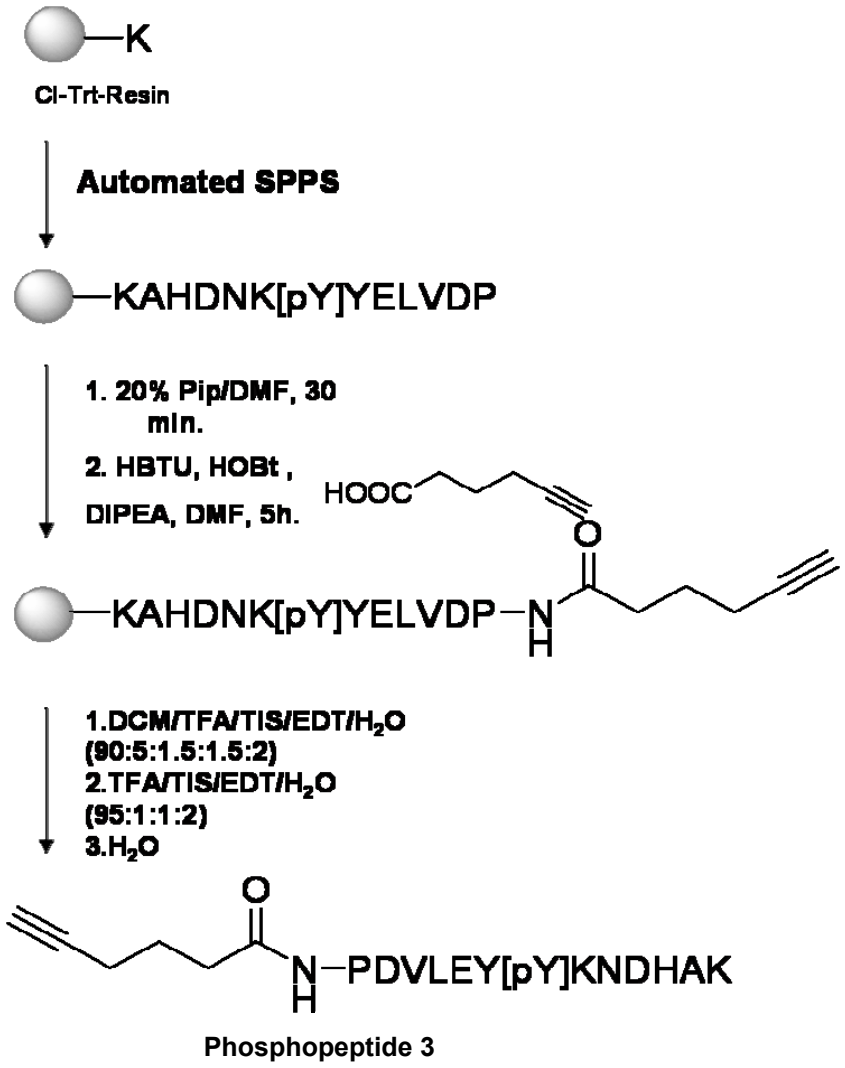
1-[N-(2-(2-hydroxyethoxy)ethyl)hex-5-ynamide]-α-D-mannoside (2):

α-D-mannose-alkyne **2** was prepared from 1,2,3,4,6-penta-*O*-acetyl-D-mannose and alkyne linker N-(2-(2-hydroxyethoxy)ethyl)hex-5-ynamide (Scheme S1) following the reported procedure.[3]

^{1}H NMR (400 MHz, *CDCl$_3$*) δppm 6.74 (1H), 4.87 (1H), 4.75 (4H), 4.00-3.71 (6H), 3.61 (3H), 3.58-3.49 (3H), 3.46-3.36 (2H), 2.34 (2H), 2.24 (2H), 2.07 (1H), 1.88-1.78 (2H): ESI-MS: 362.04 $[M + H]^+$ 384.03 $[M + Na]^+$ 200.10 [M-sugar]$^+$, calcd. 361.38 for $C_{16}H_{27}NO_8$; HR-ESI-MS: 362.1810$[M + H]^+$, calcd. 362.1809 for $C_{16}H_{28}NO_8$.

^{1}H NMR of compound 2





Scheme S2. Automated solid phase peptide synthesis (SPPS) and manual incorporation of alkyne handle.

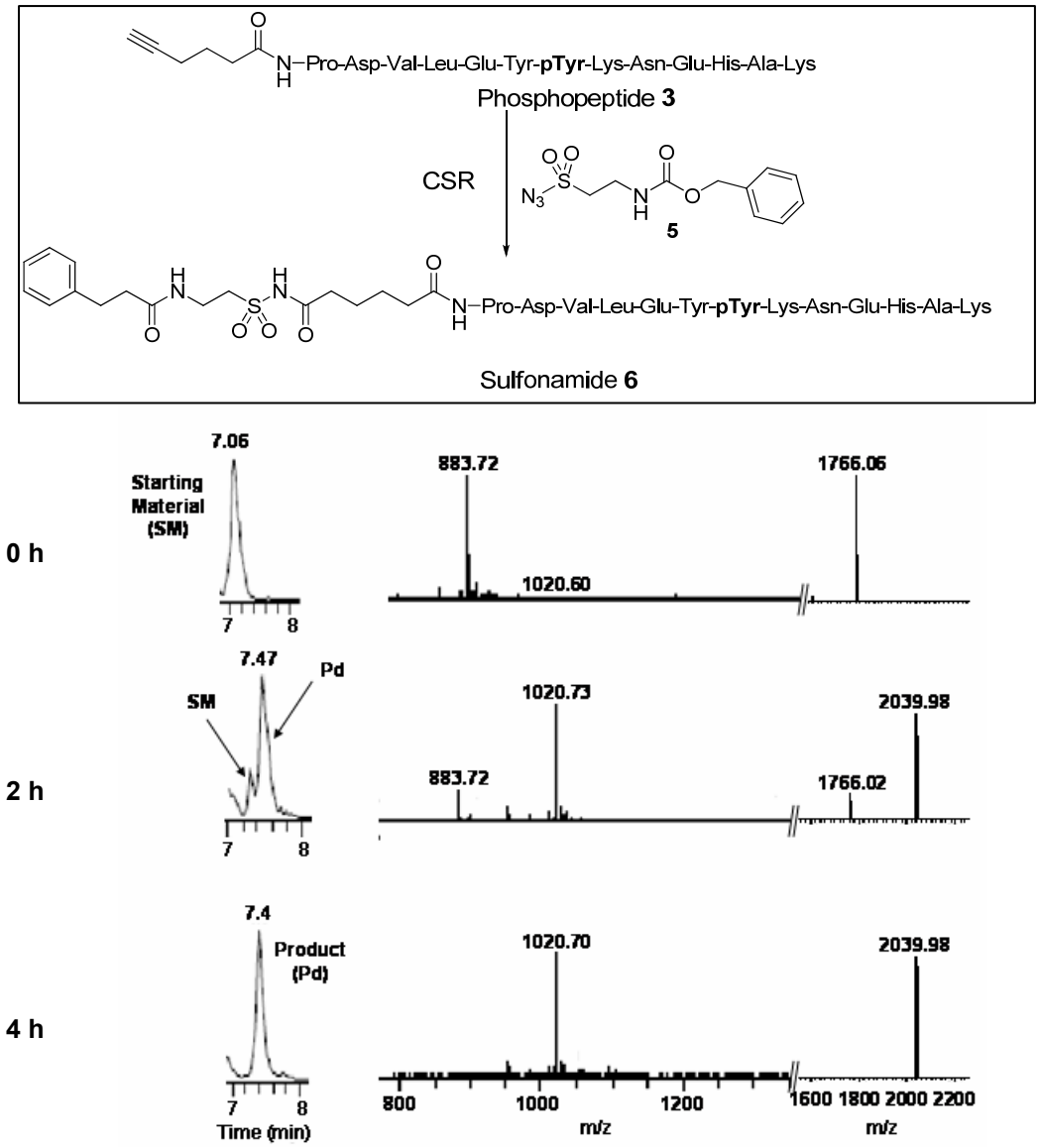


Fig. **S2 LC-MS Reaction profile**: Ligation of alkyne modified phosphopeptide **3** to a Z-tauryl sulfonylazide **5** via click sulfonamide reaction (CSR) to give sulfonamide **6** as monitored by LC-MS spectra of the reaction mixture at 0, 2 and 4h. The reaction completed at 4h. Molecular ion mass was deduced from deconvoluted LC-MS spectra. **SM**, Phosphopeptide **3,** 883.72 $[M/2]^+$, 1766.02 $[M+H]^+$; **Pd**, ligation product Sulfonamide **6**, 1020.70 $[M/2]^+$, 2039.98 $[M]^+$.

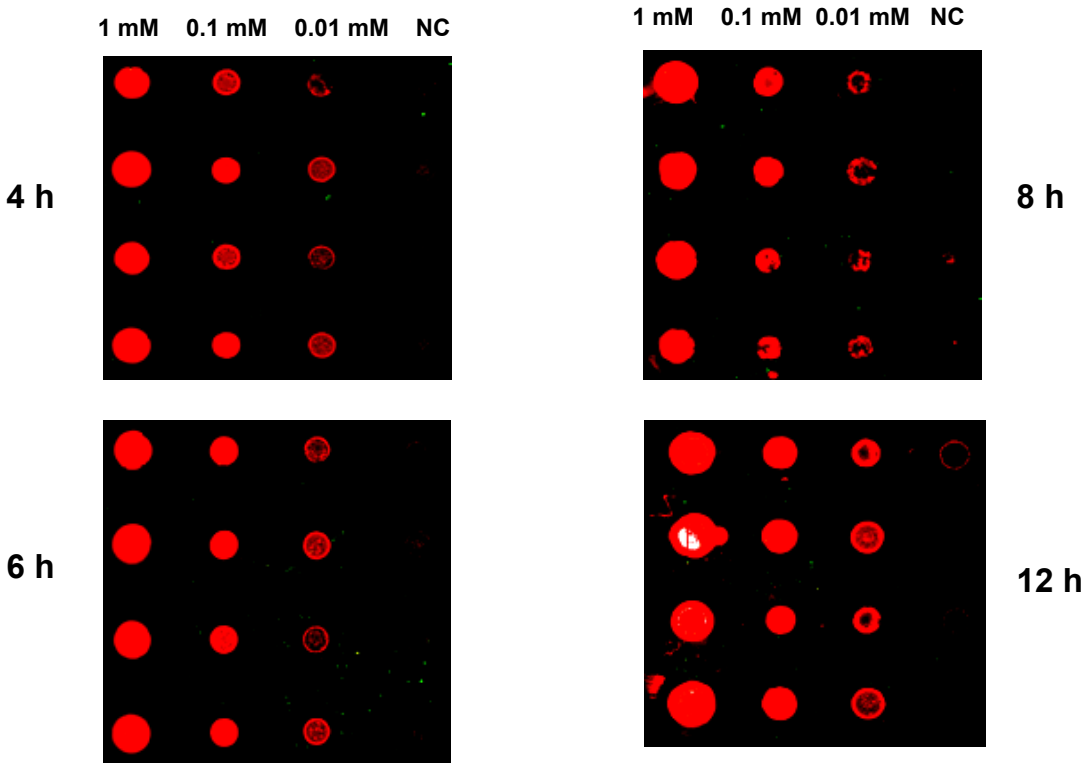


Fig. **S3** Biotin arrays: Biotin was spotted onto sulfonylazide modified dendrimer slides and the slides were stored in a humidity chamber for four different time intervals. At 4h reaction time, a good immobilization of biotin-alkyne **1** was observed after treatment with streptavidin-Cy5. NC = Biotin without alkyne (1 mM).

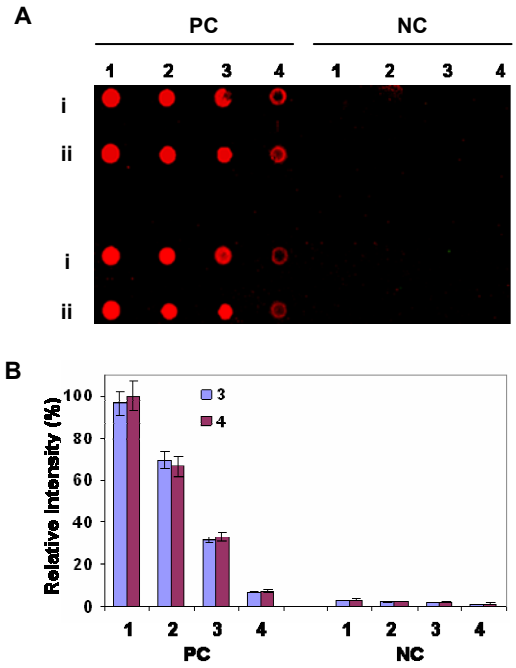


Fig. **S4 Phosphopeptide arrays**. **A**: Phosphopeptides **3** (**i**) and **4** (**ii**) were immobilized onto sulfonylazide slides via CSR at four different concentrations 1 mM (1), 0.1 mM (2), 0.01 mM (3) and 0.001 mM (4) and as duplicates. Phosphopeptides corresponding to **3** and **4** which do not possess an alkyne functionality were used as negative controls (NC). The immobilized phosphopeptides were detected by treating the slide with an anti-phosphotyrosine antibody-biotin-streptavidin-Cy5 (Ab-Biotin-Strep-Cy5) conjugate and fluorescence scanning. **B**: Plot of relative fluorescence intensity

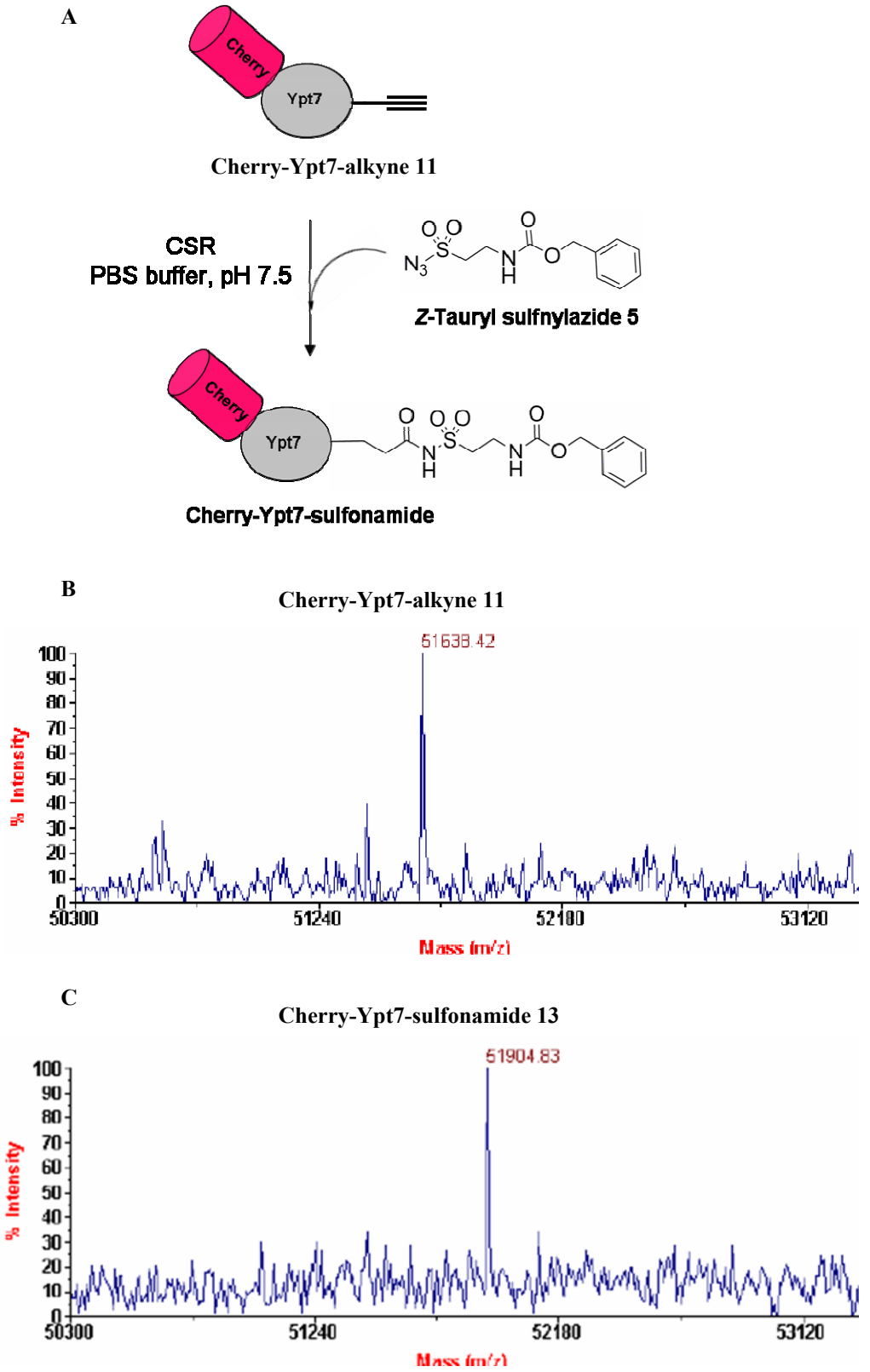


Fig. **S5 A**: Click sulfonamide ligation of Cherry-Ypt7-alkyne **11** with Z-tauryl sulfonyl azide **5**. **B** and **C**: MALDI-TOF spectra of Cherry-Ypt7-alkyne **11** and Cherry-Ypt7-sulfonamide **13** respectively.

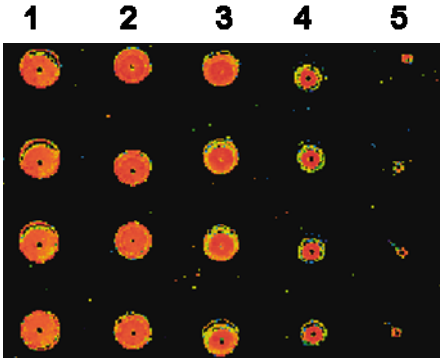


Fig. **S6 Determination of the lower detection limit**. Arrays of Cherry-Ypt7-alkyne **11** was immobilized onto sulfonyl azide modified dendrimer glass slide via click sulfonamide reaction at the following concentrations 10μM (1), 7.5μM (2), 5μM (3), 2.5μM (4), and 1μM (5). The lower detection limit of the click sulfonamide reaction based immobilization was found to be 2μM solution concentration of the protein used for spotting.

Protein expression: The RBD-thioester (Ras-binding domain of cRaf-1, aa 51-134) was generated as described before.[4] In brief the protein was expressed in *E. coli* as a intein-fusion protein. After binding to chitin beads the corresponding C-terminal thioester was generated by MESNA induced cleavage.
The GDP/GppNHp (GppNHp is a non-hydrolyzable GTP analogue) exchange for Ras (H-Ras, aa 1-180) was performed as described elsewhere.[5]

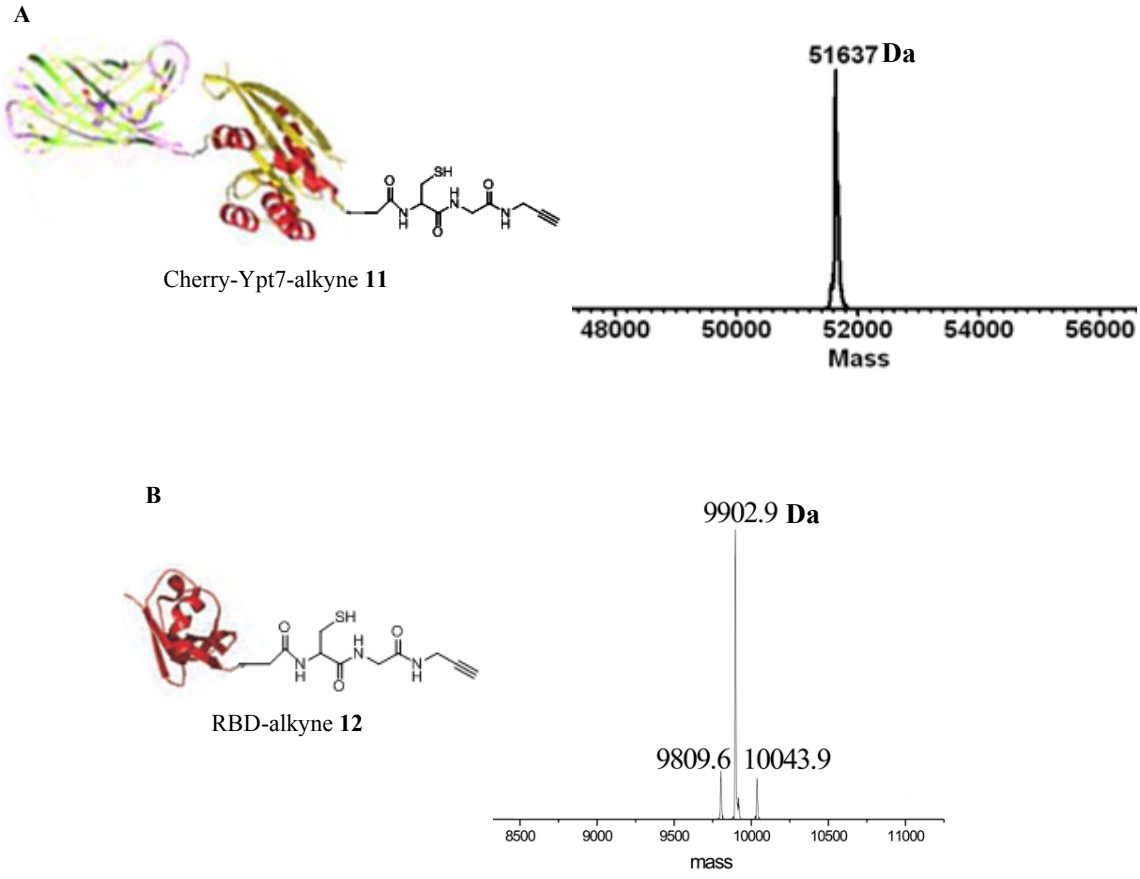


Fig. **S7 A**: Structure of Cherry-Ypt7-alkyne **11** and corresponding ESI-MS (expected MW: 51635 Da). **B**: Structure of RBD-Alkyne **12** and corresponding ESI-MS (expected MW: 9902.3 Da).

Cherry-Ypt7 (plasmid pTWIN-1-CherryYpt7-Δ3) was expressed in *E. coli* BL21(DE3)RIL cells under control of the T7 promotor. Expression was induced by 0.3 mM IPTG. After expression over night at 18ºC, cells were harvested by centrifugation. After lysis of the cells by sonification in PBS buffer and centrifugation the cytoplasmatic fraction was loaded onto chitin beads. The C-terminal thioester was formed by a MESNA induced cleavage reaction. Further purification was achieved by gel filtration.

Ligation: To obtain the RBD- and Cherry-Ypt7-alkyne 2 mg of the respective protein were mixed with 100 eq. of the dipeptide propargylamide **10** in PBS-buffer. The native chemical ligation was performed over night at 4ºC with MESNA (250 mM) as ligation mediator. After concentrating the sample, separation of the protein-alkyne and the dipeptide propargylamide **10**, was performed by gel filtration over a NAP-5 column (Amersham Biosciences).

Antibody labeling: For fluorescence readout of Ras-RBD binding the monoclonal antibody H-Ras(259) (Santa Cruz Biotechnology, Inc.) was Cy5-labeled with the Amersham Cy5 Mono-reactive Dye pack (GE Healthcare) following the instructions in the manual.

References

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