

SUPPORTING INFORMATION

BIOCOMPATIBLE SILICON SURFACES THROUGH ORTHOGONAL CLICK CHEMISTRIES AND A HIGH AFFINITY SILICON OXIDE BINDING PEPTIDE

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SUPPORTING DATA

Fragmentation of c[RGDfK]-highSP-TAMRA during MALDI-ToF-MS

In contrast to the RP-HPLC and ESI-MS which indicate high purity of the final [RGDfK]-highSP-TAMRA product (Figure 4b) we detected during MALDI-ToF-MS analysis three major signals with $m/z = 812.4$, 3830.9 and 4720.9 (Figure 4a). We propose the fragmentation reaction shown in Figure S2 and could proof successfully by MALDI-ToF/ToF-MS that the $m/z = 812.4$ Peak represents a c[RGDfK]-derivative (S2). The molecular mass of the suggested molecules fit perfectly to the observed signals in MALDI-MS. Furthermore, it is known that a Diels-Alder reaction can take a reversible course^{S1}. Since the dienophile (Repe-anhydride-lysine derivative) is a product of a Diels Alder reaction between cyclooctatetraene and maleic anhydride, a reversible Diels Alder reaction can lead to a fragmentation of this starting material or dienophile containing products^{S2}. Moreover, this would be favored due to re-aromatization of (3,4).

Tetrazine – Copper Interaction

During synthesis of c[RGDfK]-highSP-TAMRA we tried to establish a simultaneous one-pot approach with parallel DAR_{inv} and CuAAC. Whereas CuAAC worked with yields comparable to the stepwise approach no DAR_{inv} products could be identified. Since it is known that copper (I) and copper (II) can form complexes with tetrazines^{S3} we hypothesize that this is caused by a rapidly formed complex between copper and the tetrazine which inhibits the DAR_{inv}. We could confirm the formation of a tetrazine complex with increased absorbance at the tetrazine typical 520 nm maximum by UV/Vis spectroscopy when tetrazine is incubated with CuSO₄ (Figure S3). This complex formation could be diminished by addition of EDTA prior to incubation with CuSO₄. Additionally, the formed tetrazine-copper complex was degraded by subsequent addition of EDTA. This indicates a reversible nature of the complex. However, Cui et al. recently reported that incubation of copper with tetrazine at temperatures between 40°-60°C which are applied during CuAAC can lead to a hydrolysis of tetrazines^{S4}. This effect matches with the observation in the UV/VIS spectroscopy that subsequent addition of EDTA diminishes the copper-tetrazine complex but the absorption profile of tetrazine remains altered compared to pre-incubation with EDTA.

ADDITIONAL REFERENCES

- (S1) Kwart, H., and King, K. (1968) Reverse Diels-Alder or Retrodiene Reaction. *Chem. Rev.* 68, 415-447.
- (S2) Reppe, W., Schlichting, O., Klager, K., and Toepel, T. (1948) Cyclisierende Polymerisation von Acetylenen.1. Über Cyclooctatetraen. *Annalen Der Chemie-Justus Liebig* 560, 1-92.
- (S3) Kaim, W. (2002) The coordination chemistry of 1,2,4,5-tetrazines. *Coord. Chem. Rev.* 230, 127-139.
- (S4) Cui, J. H., Huang, L. F., Lu, Z. Z., Li, Y. Z., Guo, Z. J., and Zheng, H. G. (2012) Synthesis and properties of five unexpected copper complexes with ring-cleavage of 3,6-di-2-pyridyl-1,2,4,5-tetrazine by one pot in situ hydrothermal reaction. *Crystengcomm* 14, 2258-2267.

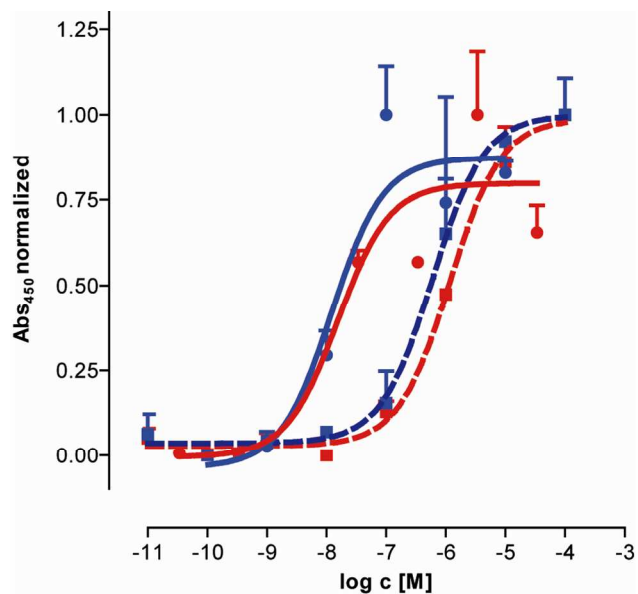


Figure S1: Biotin-ELISA: Concentration-response curves were performed for the peptides SP (dashed lines) and highSP (solid lines). The absorption signal in the biotin-ELISA of the peptides on acetone prepared silicon surfaces are presented in red. Piranha solution etched silicon surfaces are presented in blue.

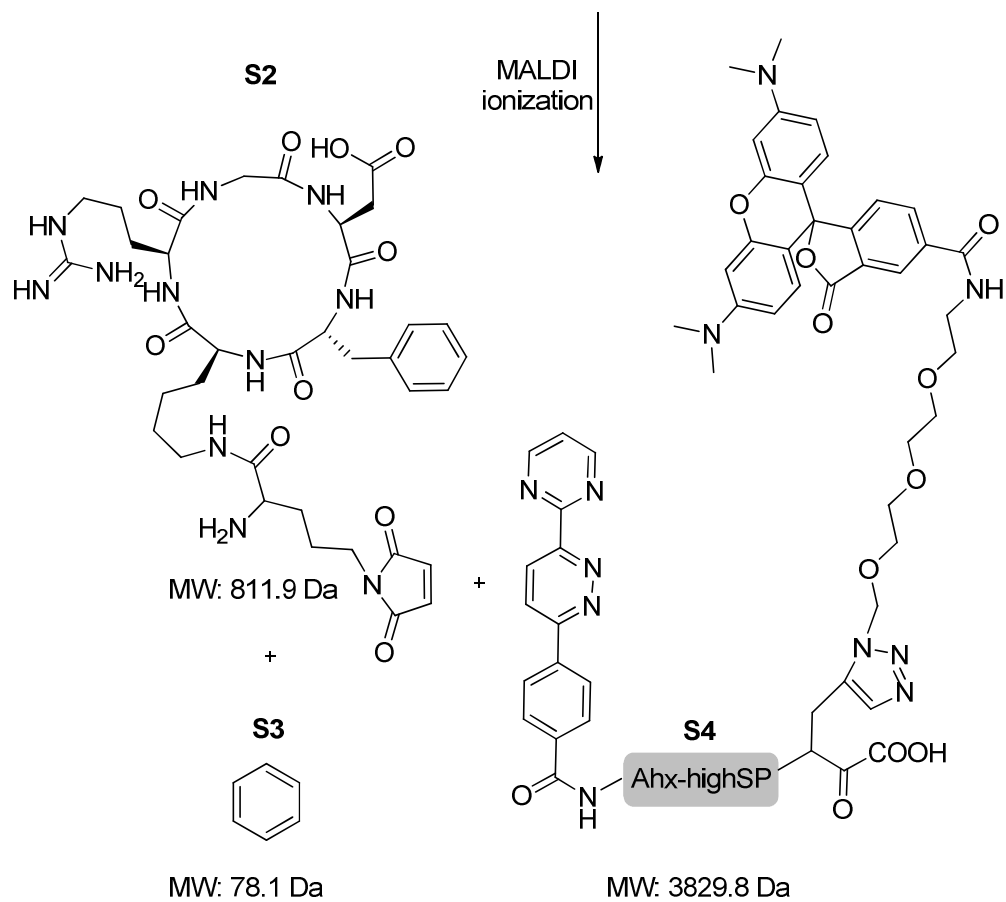


Figure S2: Putative MALDI-ToF-MS fragmentation of c[RGDfK]-highSP-TAMRA

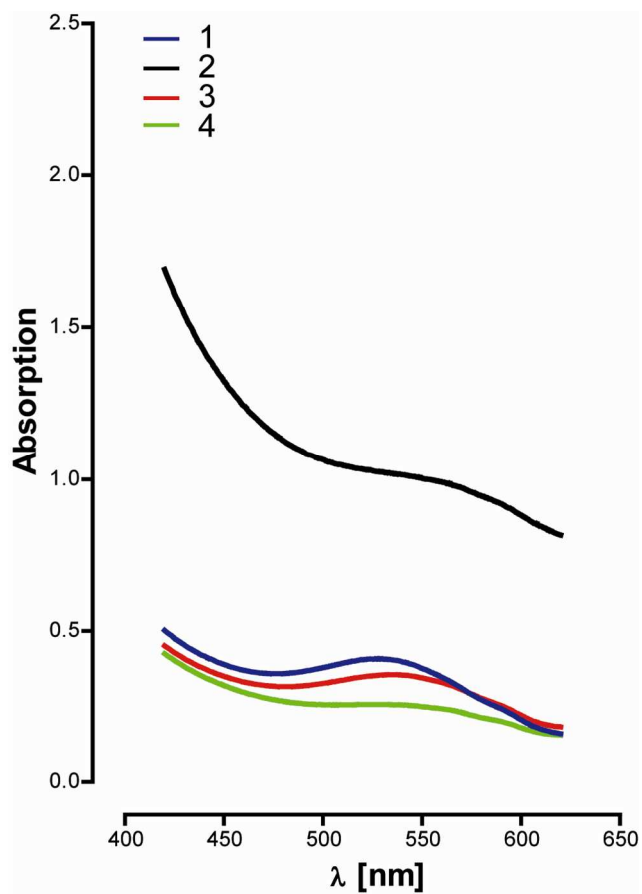


Figure S3: UV/VIS spectroscopy of the diene 1,4-diaryl-1,2,4,5-tetrazine (1 mM) after 5 min incubation with (1) 10 mM EDTA, (2) 1 mM CuSO₄, (3) 1 mM CuSO₄ and 10 mM EDTA, (4) 10 mM EDTA added after 5 min to (2). (1) Matches the absorption spectra of the diene without any additives.