Published in final edited form as:

Langmuir. 2010 June 1; 26(11): 7675–7678. doi:10.1021/la101192v.

Chemoselective Immobilization of Peptides on Abiotic and Cell Surfaces at Controlled Densities

Venkata R. Krishnamurthy¹, John T. Wilson^{1,2}, Wanxing Cui¹, XueZheng Song³, Lasanajak Yi³, Richard D. Cummings³, and Elliot L. Chaikof^{1,2,*}

¹Departments of Biomedical Engineering and Surgery, Emory University, Atlanta, GA-30322, USA

²Department of Biomedical Engineering, Emory University/Georgia Institute of Technology, Atlanta, GA-30322, USA

³Department of Biochemistry, Emory University, Atlanta, GA-30322, USA

Abstract

We report herein a new and enabling approach for decorating both living and abiotic surfaces with the extracellular matrix IKVAV peptide in a site-specific manner using strain promoted azide-alkyne cycloaddition. A cyclooctyne derivatized IKVAV peptide was synthesized and immobilized on the surface of pancreatic islets through strain-promoted azide-alkyne cycloaddition with cell surface azides generated by electrostatic adsorption of cytocompatible poly(L-lysine)-*graft*-poly(ethylene glycol) (PLL-g-PEG) copolymer bearing azido groups (PP-N₃). Both “one-pot” and sequential addition of PP-N₃ and a cyclooctyne derivatized IKVAV peptide conjugate enabled efficient modification of pancreatic islet surface in less than 60 min. The ability to bind peptides at controlled surface densities was demonstrated in a quantitative manner using microarrays. Additionally, the technique is remarkably rapid and highly efficient, opening new avenues for molecular engineering of cellular interfaces and protein and peptide microarrays.

Introduction

Re-engineering of mammalian cell surface proteomes offers a valuable tool for investigating and directing biological processes governed by cell surface molecules.¹ Genetic approaches have been commonly employed to introduce exogenous proteins or peptides to cell surfaces,^{1a-c} but many cell types and proteins may be recalcitrant to genetic manipulation.² While cell surface proteins and peptides can be introduced through covalent tethering to native cell surface constituents^{1d,e} or by insertion of membrane-anchored proteins into the lipid bilayer^{1f,g}, such processes may perturb cell physiology in unanticipated or undesired manners. Hence, a need exists for new chemical strategies to molecularly remodel cell surfaces³ where the technique is characterized by fast reaction kinetics, absence of a metal catalyst, and insensitivity towards air oxidation.⁴⁻⁸ To address these issues, Bertozzi developed a novel difluoreneated cyclooctyne whose ring strain and electron withdrawing fluorine substituents promoted [3+2] azide-alkyne cycloaddition without the need for copper catalyst.⁴ Recently, Boons and co-workers have reported a strained dibenzocyclooctynol that reacts with azides at a much higher rate.⁵ Its efficiency is comparable to that of copper catalyzed click chemistry and, thus far, has found utility in labeling cell surfaces,⁴⁻⁶ hydrogel formation⁷ and dynamic imaging of living systems.⁸

*Corresponding Author Tel: 404-727-8413; Fax: 404-727-3396; echaiko@emory.edu.

Supporting Information Available: Description of the material. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Here, we describe a new and enabling approach for immobilizing peptides using cyclooctyne promoted azide-alkyne cycloaddition (Scheme 1). First, we present a cytocompatible approach for re-engineering the surface of cells with an extracellular matrix derived peptide. Secondly, we demonstrate that strain promoted cycloaddition affords a new immobilization strategy for the generation of peptide microarrays. Here, we utilized isolated pancreatic islets, multicellular aggregates responsible for regulating blood glucose levels that have been widely explored as a cell-based therapy for the treatment of diabetes. The clinical efficacy of islet transplantation remains limited by numerous challenges, including insufficient revascularization and impaired insulin secretion,^{3a} which might be addressed through immobilizing bioactive peptides on the islet surface. Encapsulation of islets in hydrogels containing the extracellular matrix IKVAV peptide has been shown to improve islet function by inhibiting β -cell apoptosis and increasing insulin secretion^{9,10} through interaction with integrin $\alpha 6 \beta 1$ expressed on islet cell surfaces¹⁰. Therefore, IKVAV was used as a biologically relevant model peptide for functionalizing with cyclooctyne (CyO) and subsequently immobilizing on the surface of pancreatic islets.

The use of azide-cyclooctyne chemistry has notable advantages over other chemoselective cell surface engineering approaches,^{1d} most notably that it is highly efficient and that neither azide nor cyclooctyne exist naturally on the cell surface. However, a limitation of this approach is the amount of time it generally takes to generate cell surface azides through metabolic engineering approaches. Hence, the strategy reported here is remarkably rapid relative to other approaches for incorporating molecules on cell surfaces through copper-free click chemistry.^{4,5} This is anticipated to both open up new applications, particularly for cell transplant and in vivo applications, as well as to make the process of cell surface modification more convenient and less time and resource intensive.

The CyO-IKVAV conjugate can be immobilized on islets through strain promoted cycloaddition with cell surface azides generated through adsorption of a cytocompatible, poly (L-lysine)-*graft*-poly(ethylene glycol) (PLL-*g*-PEG) copolymer bearing azido groups (PP-N₃).⁶ To gain further insight into this strategy, CyO-IKVAV was conjugated to surface-bound PP-N₃ in a microarray format. Significantly, we observed that peptide surface density could be tailored through control of peptide concentration and copolymer structure.

Results and Discussion

In a recent report, cyclooctyne was conjugated to peptides using amine groups⁷ (e.g., lysine residues and N-terminus) as the reactive nucleophilic partner. This strategy would be anticipated to interfere with the activity of IKVAV and other lysine-containing peptide sequences. Hence, an N-terminal cysteine was incorporated in the IKVAV sequence to enable site-specific incorporation of cyclooctyne through a thiol conjugation strategy. Additionally, two glycine spacers and a Lys (Biotin) amino acid were introduced in the sequence to facilitate peptide detection with fluorescently labeled streptavidin.

Cyclooctyne derivatives cannot be directly incorporated to a resin bound peptide owing to the instability of this group to trifluoroacetic acid and other scavengers, which are applied during peptide cleavage from the resin.¹¹ Thus, IKVAV was first synthesized on the resin and, subsequently, the cyclooctyne derivative was attached using a chemoselective ligation reaction. Although various cysteine functionalization strategies such as thiol-ene reaction¹² and allylic disulfide rearrangement¹³ can be applied to introduce the cyclooctyne derivative in the IKVAV peptide sequence, we utilized a thiol-maleimide¹⁴ conjugation strategy in the present investigation. Our strategy to introduce cyclooctyne in the IKVAV peptide sequence is shown in Scheme 2. Boons cyclooctyne **1**⁵ was first activated and subsequently coupled to *N*-(2-aminoethyl) maleimide to afford the cyclooctyne derived heterobifunctional linker **3** in

42% yield. Thiol-maleimide conjugation of linker **3** to CK(Biotin)-GGIKVAV gave the target CyO-IVAV peptide in 71% yield (Scheme 2).

To generate azide groups on the islet surface,⁶ islets were incubated in PP-N₃ (~40% grafted, 80 μ M, 40 min), rinsed to remove non-adsorbed copolymer, and incubated in CyO-IKVAV (100 μ M, 1 h). Non-bound peptide was washed away and islets subsequently incubated in Cy3-labeled streptavidin (Cy3-SA, 0.1 mg/ml) for 30 minutes to detect the biotin group in the peptide sequence. IKVAV could be immobilized on the surface of individual islets in a cyclooctyne- and azide-specific manner (Figure 1). Peptide was presented on both peripheral cells, as well as those within the core of the islet yielding a honeycomb-like pattern of fluorescence. Importantly, islet viability was preserved upon treatment with PP-N₃ and CyO-IKVAV (100.4 $\pm$ 1.25% viable relative to untreated controls; see Supporting Information for representative micographs), a testament to the cytocompatibility of both appropriately structured PLL-g-PEG copolymers and copper-free click chemistry.

Of note, “one-pot” modification of islets with peptide could be achieved through concomitant copolymer adsorption and chemoselective ligation of the peptide by simultaneously adding PP-N₃ and CyO-IKVAV to islets. To our knowledge, this represents the *first* report to describe the molecular engineering of cell-supported extracellular matrices. Moreover, as a wide variety of cyclooctyne-modified peptides can be immobilized in this manner, this strategy provides a highly modular platform for re-engineering cellular interfaces with matrix constituents.

The surface density of cell adhesive peptides, such as IKVAV, can have a significant effect on integrin-dependent cell behavior, including adhesion, differentiation, and survival.^{15,16} To explore the facility of this strategy to tailor peptide surface concentration, a microarray approach was utilized, which allowed process variables to be investigated in a rapid and quantitative manner, otherwise not plausible using islets. Additionally, microarrays in which peptides are immobilized to a surface would enable high-throughput screening that requires only small quantities of analyte.¹⁷

As a model surface, we chose an amine-reactive NHS slide which would rapidly couple PP-N₃ to the substrate through non-modified backbone lysine residues. First, serial dilutions (25 μ M, 12 μ M, 6 μ M and 3 μ M and 1.5 μ M) of PP-N₃ synthesized with PEG chains of n=4 or 12 monomers (PP4-N₃ and PP12-N₃, respectively) were printed on NHS activated glass surfaces using a piezoelectronic non-contact printer. Biotin-hydrazine (50 μ M) was printed as a fluorescence intensity standard. Each concentration was printed as six duplicate spots by depositing 0.33 nL of linker solution yielding an average spot size of ~100 μ m. After printing, the slide was incubated at 50°C for 1 hour in a moisture controlled chamber and then at room temperature for 12 h to ensure complete reaction. The slide was then thoroughly washed with buffer and then blocked with 50 mM aqueous ethanolamine to prevent the non-specific absorption of the primary amines. Strain promoted azide-alkyne cycloaddition was performed by incubating the slide with CyO-IKVAV at 50 and 25 μ M concentrations in phosphate buffer for 15 min. Detection of immobilized peptide was accomplished by incubating the slide with Alexa488-streptavidin (5 μ g/mL) for 1 h followed by fluorescence detection at 495 nm/519 nm (Ex/Em) with a Perkin Elmer ProScanarray microarray scanner (Figure 2).

Immobilization on surface microarrays by strain promoted cycloaddition is efficient and quantification of fluorescence intensity indicated that peptides could be immobilized on the solid support in less than 15 minutes. Control experiments (Figure 2C) confirmed that binding was dependent on strain promoted cycloaddition. Surface-bound peptide density could be controlled by adjusting the concentration of either PP-N₃ or the CyO-peptide conjugate. As seen in Figure 3, PP-N₃ synthesized with PEG₄ spacer (PP4-N₃) afforded surfaces with higher levels of immobilized peptide relative to structural analogs synthesized with PEG₁₂ spacers

(PP12-N₃). Presumably, diminished steric effects due to the shorter chain length of PP4-N₃ variant in the intermediate NHS coupling step supported enhanced immobilization. Consequently, greater binding of PP4-N₃ was observed in the subsequent strain promoted azide-alkyne cycloaddition.

In summary, we report a novel approach to immobilizing peptides both on cellular and solid surfaces in a site-specific and chemoselective manner through strain promoted azide-alkyne cycloaddition. In contrast to other peptide immobilization strategies, this method is robust under physiological conditions and does not require a metal catalyst, allowing the surface of viable mammalian cells and tissues to be decorated with peptide sequences without compromising cell viability. Additionally, this approach will overcome the limitations of conventional methods for surface bioconjugation, which are limited by low efficiency, selectivity and harsh reaction conditions. Collectively, this work provides a versatile platform for immobilizing peptides and other CyO containing biomolecules on both abiotic and cell surfaces with multiple potential biomedical and biotechnological applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grants from National Institutes of Health (DK069275) and the Juvenile Diabetes Research Foundation. JTW acknowledges the Whitaker Foundation for generous fellowship support.

REFERENCES

- (1). (a) Fernandez-Suarez M, Baruah M, Martinez-Hernandez L, Xie KT, Baskin JM, Bertozzi CR, Ting AY. *Nat. Biotechnol* 2007;25:1483–1487. [PubMed: 18059260] (b) Bonasio R, Carman CV, Kim E, Sage PT, Love KR, Mempel TR, Springer TA, von Andrian UH. *Proc. Natl. Acad. Sci. U.S.A* 2007;104:14753–14758. [PubMed: 17785425] (c) Keppler A, Gendreizig S, Gronemeyer T, Pick H, Vogel H, Johnsson K. *Nat. Biotechnol* 2003;21:86–89. [PubMed: 12469133] (d) Sarkar D, Vemula PV, Teo GSL, Spelke D, Karnik R, Wee LY, Karp JM. *Bioconjugate Chem* 2008;19:2105–2109. (e) Stabler CL, Sun XL, Cui W, Wilson JT, Haller CA, Chaikof EL. *Bioconjug. Chem* 2007;18:1713–1715. [PubMed: 17960873] (f) Boonyarattanakalin S, Martin SE, Sun Q, Peterson BR. *J. Am. Chem. Soc* 2006;128:11463–11470. [PubMed: 16939269] (g) Ko IK, Kean TJ, Dennis JE. *Biomaterials* 2009;30:3702–3710. [PubMed: 19375791]
- (2). (a) Rabuka D, Forstner MB, Groves JT, Bertozzi CR. *J. Am. Chem. Soc* 2008;130:5947–5953. [PubMed: 18402449] (b) Mahal LK, Bertozzi CR. *Chem. Biol* 1997;4:415–422. [PubMed: 9224572]
- (3). (a) Wilson JT, Chaikof EL. *Adv. Drug. Deliv. Rev* 2008;60:124–145. [PubMed: 18022728] (b) Totani T, Teramura Y, Iwata H. *Biomaterials* 2008;29:2878–2883. [PubMed: 18395793] (c) Dwyer KM, Mysore TB, Crikis S, Robson SC, Nandurkar H, Cowan PJ, D'Apice AJ. *Transplantation* 2006;82:428–432. [PubMed: 16906044]
- (4). (a) Agard NJ, Prescher JA, Bertozzi CR. *J. Am. Chem. Soc* 2004;126:15046–15047. [PubMed: 15547999] (b) Codelli JA, Baskin JM, Agard NJ, Bertozzi CR. *J. Am. Chem. Soc* 2008;130:11486–11493. [PubMed: 18680289]
- (5). Ning X, Guo J, Wolfert MA, Boons G-J. *Angew. Chem, Int. Ed* 2008;47:2253–2255.
- (6). Wilson JT, Krishnamurthy V, Cui W, Qu Z, Chaikof EL. *J. Am. Chem. Soc* 2009;131:18228–18229. [PubMed: 19961173]
- (7). De Forest CA, Polizzotti BD, Anseth KS. *Nat. Mater* 2009;8:659–664. [PubMed: 19543279]
- (8). Laughlin ST, Baskin JM, Amacher SL, Bertozzi CR. *Science* 2008;320:664–667. [PubMed: 18451302]

- (9). Weber LM, Hayda KN, Haskins K, Anseth KS. *Biomaterials* 2007;28:3004–3011. [PubMed: 17391752]
- (10). Weber LM, Anseth KS. *Matrix Biol* 2008;27:667–663. [PubMed: 18773957]
- (11). Kele P, Mezo G, Achatz D, Wolfdeis OS. *Angew. Chem., Int. Ed* 2009;48:344–347.
- (12). (a) Geng Y, Discher DE, Justynska J, Schlaad H. *Angew. Chem., Int. Ed* 2006;45:7578–7581. (b) Hordyjewicz-Baran Z, You L, Smarsly B, Sigel R, Schlaad H. *Macromolecules* 2007;40:3901–3903. (c) Triola G, Brunsveld L, Waldmann H. *J. Org. Chem* 2008;73:3646–3649. [PubMed: 18376863]
- (13). Crich D, Krishnamurthy V, Hutton TK. *J. Am. Chem. Soc* 2006;128:2544–2545. [PubMed: 16492032] (b) Crich D, Brebion F, Krishnamurthy V. *Org. Lett* 2006;8:3593–3596. [PubMed: 16869668] (c) Crich D, Krishnamurthy V, Brebion F, Karatholuvhu M, Subramanian V, Hutton TK. *J. Am. Chem. Soc* 2007;129:10282–10294. F. [PubMed: 17655306] (d) Crich D, Yang FJ. *J. Org. Chem* 2008;73:7017–7027. [PubMed: 18729514]
- (14). (a) Smyth DG, Blumenfeld OO, Konigsberg W. *Biochem. J* 1964;91:589–595. [PubMed: 5840721] (b) Gorin G, Martin PA, Dougherty G. *Arch. Biochem. Biophys* 1966;115:593–597. [PubMed: 5970483] (c) Partis MD, Griffiths DG, Roberts GC, Beechey RB. *J. Protein. Chem* 1983;2:263–277.
- (15). Thid D, Bally M, Holm K, Chessari S, Tossatti S, Textor M, Gold J. *Langmuir* 2007;23:11693–11704. [PubMed: 17918863]
- (16). Hersel U, Dahmen C, Kessler H. *Biomaterials* 2003;24:4385–4415. [PubMed: 12922151]
- (17). (a) Kodadek T. *Chem. Biol* 2001;8:105–115. [PubMed: 11251285] (b) Zhu H, Snyder M. *Curr. Opin. Chem. Biol* 2001;5:40–45. [PubMed: 11166646] (c) Lee Y–S, Mrksich M. *Trends Biotech* 2002;20:S14–S18.
- (18). Sun X–L, Yang L, Chaikof EL. *Tetrahedron Lett* 2008;49:2510–2513. [PubMed: 19568325]

Figure 1.

(A) Immobilization of cyclooctyne derivatized IKVAV peptide on islet surfaces (left); Representative confocal micrograph of islets serially treated with PP-N₃ and CyO-IKVAV followed by detection with Cy3-streptavidin (right). (B) Control Experiments demonstrate negligible non-specific binding of the peptide to islet surfaces. PP-N₃+ control Peptide 4 + Cy3-SA (left); PP-(OCH₃) + CyO-IKVAV + Cy3-SA (right). Scale bar 50 μ m. (C) Islet viability of demonstrates that treatment of PP-N₃+CyO-IKVAV do not significantly ($p>0.05$) influence islet viability relative to untreated controls.

Figure 2.

Fluorescence micrograph of PP4-N₃ and PP12-N₃ printed at 25 μ M, 12 μ M, 6 μ M, 3 μ M and 1 μ M. Each concentration was spotted with six replicates, vertically from top left to bottom right with biotin-hydrazine control in the lower right corner. (A) PP-N₃ + CyO-IKVAV (50 μ M); (B) PP-N₃ + CyO-IKVAV (25 μ M); (C) PP-N₃ + control peptide **4** (50 μ M).

Figure 3. Normalized Relative Fluorescence intensities of CyO-IKVAV peptides immobilized by strain promoted cycloaddition. PLL-g-PEG copolymers presented N₃ at the terminus of PEG₄ (PP4-N₃) or PEG₁₂ (PP12-N₃) spacers incorporated at a similar grafting ratio (40-45%).

Scheme 1.

Peptide immobilization on abiotic and living surfaces using strain promoted azide-alkyne cycloaddition

Scheme 2.

Synthesis of CyO-IKVAV using Thiol-Maleimide Conjugation

Reagents and Conditions. a) 4-nitrophenylchloroformate, pyridine, CH_2Cl_2 , 70 % b) *N*-2-aminoethylmaleimide trifluoroacetate salt, DIPEA, CH_3CN , 42 % c) CK(Biotin)GGIKAV **4**, phosphate buffer, pH 7.5, 71 %.