One-Step Enzymatic Modification of the Cell Surface Redirects Cellular Cytotoxicity and Parasite Tropism

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Supporting Information

ABSTRACT: Surface display of engineered proteins has many useful applications. The expression of a synthetic chimeric antigen receptor composed of an extracellular tumor-specific antibody fragment linked to a cytosolic activating motif in engineered T cells is now considered a viable approach for the treatment of leukemias. The risk of de novo tumor development, inherent in the transfer of genetically engineered cells, calls for alternative approaches for the functionalization of the lymphocyte plasma membrane. We demonstrate the conjugation of LPXTG-tagged probes and LPXTG-bearing proteins to endogenous acceptors at the plasma membrane in a single step using sortase A. We successfully conjugated biotin probes not only to mouse hematopoietic cells but also to yeast cells, 293T cells, and Toxoplasma gondii. Installation of single domain antibodies on activated CD8 T cells redirects cell-specific cytotoxicity to cells that bear the relevant antigen. Likewise, conjugation of Toxoplasma gondii with single domain antibodies targets the pathogen to cells that express the antigen recognized by these single domain antibodies. This simple and robust enzymatic approach enables engineering of the plasma membrane for research or therapy under physiological reaction conditions that ensure the viability of the modified cells.

RESULTS AND DISCUSSION

Engineering of the Cell Surface in Absence of Genetic Modification Using Sortase A. We and others have used sortase A from Staphylococcus aureus to conjugate modified probes onto the C-terminus of recombinant LPETG-tagged proteins, in a process referred to as "sortagging" (Figure 1a).20,21 The reaction proceeds as follows: sortase attacks the LPETG tag to cleave between T and G with concomitant formation of a covalent acyl-enzyme intermediate between sortase and the tagged protein.22,23 The covalent acyl-enzyme intermediate is resolved by a nucleophilic attack, using an amino acid with an N-terminal Gly residue(s) to the protein or peptide containing an LPTEG motif.18,19 As described below, we show that LPTEG-tagged probes and proteins can be conjugated using sortase A in a single step to glycines naturally exposed at the cell surface. We show that the conjugation of single domain antibodies to CD8 T cells and to Toxoplasma can redirect specific cytotoxicity and infection, respectively.
at its NH2-terminus. This method can be applied to the modification of type II proteins on the surface of cells through the genetic insertion of a C-terminal sortase recognition tag. In a conceptually similar fashion, two di

cations, we

Redirection of T Cell Cytotoxicity through Surface Conjugation of Single Domain Antibodies. Expression of tumor-specific chimeric antigen receptors at the surface of CD8 T cells is emerging as practical approach to eradicate leukemic cells. To investigate whether each subset was subject to modification, we monitored the installation of biotin-LPETG by flow cytometry, using fluorescently labeled streptavidin together with cell type-specific antibodies. Biotin-LPETG probes labeled T and B cells equally well and erythrocytes slightly less efficiently (Figure 1h). We measured by flow cytometry the kinetics with which biotin-LPETG was conjugated to erythrocyte-depleted splenocytes. Conjugation reached ~30% of maximum after 5 min and ~60% of maximum after 15 min (Figure 1i). Collectively, our data show that all cells tested were efficiently sortagged in a timeframe compatible with biological experiments. Presumably most cells have naturally exposed glycines at their cell surface and will therefore be amenable to sortagging.

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LPETG to the reaction for 15 min (Figure 2c). Cells incubated with enhancer-LPETG prior to biotin-LPETG had similar amounts of surface-conjugated biotin compared to cells incubated with biotin-LPTEG alone (Figure 2c). These data suggest that sortagging of VHHs to cells only minimally affects subsequent conjugation of biotin-LPETG. The smaller LPETG-tagged probes may have more ready access to surface-displayed nucleophiles left unoccupied by larger LPETG-tagged proteins.

We next addressed whether the conjugation of VHHs to activated OTI T cells enables cell-specific killing toward cells that bear the antigen for which the VHH is specific. Co-incubation of preactivated OTI T cells sortagged using the anti-class-II MHC VHH7 with mouse splenocytes resulted in specific killing of class II MHC-positive B cells but not class II MHC-negative CD4 T cells (Figure 2d). Specific cytotoxicity showed a direct correlation with the number of conjugated VHHs. VHHs can thus be efficiently sortagged onto T cells with full retention of the VHH’s antigen binding capacity. The sortagging reaction preserved cytotoxic functions and allowed redirected killing based on VHH specificity, in a fashion analogous to, but conceptually distinct from CAR T cells (Figure 2e and f). We have installed 2 different probes on the surface of lymphocytes (Figure 2c), and there is no reason to assume that two is the limit to the number of different proteins that can be attached to T cells in this manner. Our approach opens new possibilities in the functionalization of (T) lymphocytes with cytotoxic and targeting moieties to achieve enhanced and specific tumor killing.
This enzymatic approach has several key advantages over current methods. First, because this method does not rely on genetic manipulation of the lymphocyte, it reduces genetic hazards and simplifies the process of functionalization of virtually any cell type. Second, the extent of modification can be controlled, and last, the temporary nature of these modifications, dictated by the lifespan of individual activated lymphocytes, may help to prevent undesired chronic response against healthy cells that express the targeted antigen.

**Cell-Specific Targeting of Toxoplasma gondii.** The modification of CD8 T cells through sortagging does not obviously interfere with cytotoxic functions (Figure 2d). We decided to extend these methods to the manipulation of other cell—cell interactions. To investigate whether modified T. gondii tachyzoites would still be able to invade host cells, we sortagged parasites with TAMRA-modified LPETG peptides and incubated them together with human fibroblasts. Sortagged parasites visualized by fluorescence microscopy were perfectly capable of invading fibroblasts (Figure 3a and Supporting Information Movie 1). The image shows an intracellular parasite with a distinctly labeled plasma membrane (yellow arrow) and a parasite in the process of invasion characterized by a bright arrow pointed at the constricted moving junction (white arrows). To address whether T. gondii could be targeted to specific cells, we sortagged parasites with biotin, biotin plus enhancer or VHH7, followed by incubation with WT splenocytes. Sortagging of VHH7 to T. gondii not only resulted in a dramatic increase of B cells targeted by the parasite together with a significant decrease of binding to non-B cells (Figure 3b) but also enhanced the percentages of B cells lysed upon infection (Figure 3c). The possibility of targeting genetically engineered cytolytic pathogens to cancer cells has been explored by others. In principle, the approach described here lends itself to similar strategies without the need of genome modifications: this approach is compatible not only with the majority of Gram-positive bacteria that already use sortase enzymes but also with parasites (Figure 3) and possibly with viruses.

Regardless of the utility of sortagging to endow lymphocytes with novel recognition specificities for therapeutic applications, the presence of endogenous nucleophiles also provides a mild enzymatic method to install any entity of choice on the surface of a living cell without the need for genetic modification or the use of harsh chemical conditions. Identification at the molecular level of the substrates to which the label is attached may not be required, and the ease of surface modification under mild and physiological conditions compares favorably with methods that employ radioisotopes or those requiring reaction conditions that ensure reactivity of chemical tags with primary amines or thiols at the cell surface. Sortase-based modifications can be performed in protein-containing buffers and at pH values that would preclude the use of standard chemical labeling strategies.

**METHODS**

**Mice.** C57BL/6, class II MHC deficient mice and OTI RAG deficient mice were purchased from Jackson laboratory, bred in the animal facility of the Whitehead Institute for Biomedical Research (Cambridge, MA) and maintained according to protocols approved by the MIT Committee on Animal Care.

**Cell Culture.** Erythrocytes cells were depleted from total mouse splenocytes using red blood cell lysis buffer (Sigma, cat. R7757). Mouse lymphocytes were cultured in RPMI 1640 (cat. 11875; Gibco) supplemented with 10% (v/v) inactivated FCS (Gibco), 0.0002% β-mercaptoethanol final (Sigma, cat. M7522), penicillin 50 units per liter—streptomycin 50 mg per liter (Sigma, cat. P4333), 1 mM sodium pyruvate final (Gibco, cat. 11360) nonessential amino acids (Life Technologies, cat. 11140) and 2 mM glutamine (US Biological, cat.

**Figure 3.** Toxoplasma gondii sortagging. (a) Toxoplasma gondii tachyzoites were incubated with 500 μM TAMRA-LPETG and 20 μM sortase A for 15 min. Parasites were then washed and incubated with human foreskin fibroblasts. Images show the juxtaposition of bright and fluorescent fields. Yellow arrow: intracellular parasite. White arrows: invading parasite. Scale bar: 10 μm. (b) Toxoplasma gondii tachyzoites were incubated with or without 50 μM enhancer- or VHH7-LPETG and 20 μM sortase A. After 20 min, biotin-LPETG was added for 15 min. Parasites were then washed and incubated with red-cell depleted splenocytes for 1 h at a multiplicity of infection of 5. Cells were then washed and stained with a CD19-specific antibody and fluorescently labeled streptavidin. The histogram shows the percentage of sortagged T. gondii (T.g.) positive cells within CD19 negative or positive populations. Error bars: standard deviation (n = 3). (c) Purified B cells from WT or class II MHC knock out (k.o.) mice were incubated together with control T. gondii or T. gondii sortagged with enhancer or VHH7 at a multiplicity of infection of 5. Fifteen hours after infection, cell lysis was measured and normalized to uninfected (0%) and detergent-lysed B cells (100%). Error bars: standard deviation (n = 3). **p < 0.01 at Student t test.
G7120). HEK 293T cells were cultured in Dulbecco’s Modified Eagle’s Medium supplemented with 10% (v/v) inactivated FBS ( Gibco). Saccharomyces cerevisiae strain W303 was cultured in YPD medium. Toxoplasma gondii (RH strain) tachyzoites were grown in human foreskin fibroblasts (HFF) cultured in Dulbecco’s Modified Eagles Medium (DMEM; Invitrogen) supplemented with 10% tetracycline-free FBS (HyClone), 2 mM glutamine, 10 mM HEPES (pH 7.5), and 20 μg/ml gentamicin.

Antibodies and Reagents. Anti-PGK (clone 22C5D8, Invitrogen), antimouse/human actin (clone Ab-5, BD biosciences), anti-Toxoplasma gondii actin,39 horseradish peroxidase-conjugated goat antirabbit Ig (Southern Biotech, cat. 4041-05), horseradish peroxidase-conjugated antimouse Ig (GE Healthcare, cat. NXX931), anti-TCRβ (clone H57, BD Pharmingen), anti-CD4 (clone GK1.5, ebiosciences), anti-CD19 (clone 1D4, BD Pharmingen), anti-TER119 (clone TER-119, BD Pharmingen), allophycocyanin-conjugated streptavidin (ebiosciences, cat. number 17-4317), phycoerythrin-conjugated streptavidin (Southern Biotech, cat. number 7100-09S). Propidium iodide (Sigma-Aldrich, cat. number P4864). B cell isolation kit (Miltenyi, cat. 130-090-862). For the experiments described here, we used a modified form of sortase A from Staphylococcus aureus that lacks the first 59 residues, and with the following mutations: E105 K/E108A and P94R/D160N/D165A/K190E/K196T.24 Sortase A was produced and purified as described elsewhere.34 Enhancer54 and VHFF54 were produced and purified as described elsewhere.34 Biotin-LPETG (Biotin-aminohexanoic acid-LPETGG) was produced by the MIT biopolymer facility through standard solid phase peptide synthesis. TAMRA-LPETG was produced as described elsewhere.20

Cell Sorting. Unless described otherwise, reactions were performed at room temperature (RT) for 1 h in PBS or in HHE buffer (Hanks Balanced Salt Solution, 1 mM EDTA, 25 mM HEPES pH 7). Sortase A was used at a final concentration of 20–40 μM and LPETG substrates at 500 μM unless indicated otherwise. Mouse splenocytes or lymphocytes were used at 20–100 million cells per milliliter, HEK 293T cells at 20 million per milliliter, Toxoplasma gondii at 200 to 400 million per milliliter, yeast at 6 OD 280 units per milliliter. For biochemical analysis, enzymatically modified cells were washed twice with PBS after sortagging and lysed in 1× reducing Laemmli sample buffer.

In Vitro Cytotoxicity Assay. Pooled lymph nodules cells and erythrocytes-depleted splenocytes from OT1 rag deficient mice were seeded in complete RPMI at 2 million cells per milliliter in 24 well plate previously coated with 2 μg/ml anti-CD3 (clone 17A2) and 2 μg/ml anti-CD28 (clone 37–51). After 72 h, cells were washed twice with PBS and sortaged for 1 h in PBS as described above and the figure legends. Cells were then washed once with PBS and once with complete RPMI. 1–2 × 10^5 sortaged or control cells were incubated together with 2 × 10^7 WT splenocytes in complete RPMI in U bottom 96 well plates. After 16 to 20 h the cells the percentage of living propidium iodide negative CD4 positive and CD19 positive cells were measured by flow cytometry using fluorescently labeled antibodies specific for CD4 and CD19 together with propidium iodide (final concentration 1 μg/ml).

Toxoplasma gondii Invasion Assay. Toxoplasma gondii tachyzoites were incubated with 500 μM TAMRA-LPETG and 20 μM sortase A at RT for 15 min in HHE buffer. Parasites were then washed and incubated with HFF. Parasite targeting to class II MHC positive cells: Toxoplasma gondii tachyzoites were incubated with or without 50 μM enhancer- or VHFF-LPETG and 20 μM sortase A at RT in HHE buffer. After 20 min, biotin-LPETG was added for 15 min. Parasites were then washed and incubated with red-cell depleted splenocytes for 1 h at a multiplicity of infection of 5. Cells were then washed and stained with a CD19-specific antibody and fluorescently labeled streptavidin. Cells were fixed (BD Cytofix/Cytoperm, cat. 554722) prior to analysis by flow cytometry. B cells lysis assay: Toxoplasma gondii tachyzoites were incubated with or without 50 μM enhancer- or VHFF-LPETG and 20 μM sortase A at RT in HHE buffer for 15 min. After washing T. gondii was incubated together with 0.5 million magnetic beads-purified splenic B cells from WT or class II MHC k.o. at a multiplicity of infection of 5 in 100 μl or complete RPMI in 96 flat bottom well plates. After 15 h supernatants were harvested and cell lysis was measured using CytoTox 96 Non-Radioactive Cytotoxicity Assay kit (Promega, cat. G781) according to manufacturer’s instructions.

ASSOCIATED CONTENT

Supporting Information
Table 1, Movie 1, and additional methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare the following competing financial interest(s): Hidde L. Ploegh is a founder of 121 Bio.

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REFERENCES

(1) Mahal, L. K., Yarema, K. J., and Bertozzi, C. R. (1997) Engineering chemical reactivity on cell surfaces through oligosaccharide biosynthesis. Science 276, 1125–1128.
(2) Prescher, J. A., Dube, D. H., and Bertozzi, C. R. (2004) Chemical remodelling of cell surfaces in living animals. Nature 430, 873–877.
(3) Chang, P. V., and Bertozzi, C. R. (2012) Imaging beyond the proteome. Chem. Commun. 48, 8864.
(4) Jao, C. Y., Roth, M., Welti, R., and Salic, A. (2009) Metabolic labeling and direct imaging of choline phospholipids in vivo. Proc. Natl. Acad. Sci. U.S.A. 106, 15332–15337.
(5) Neef, A. B., and Schultz, C. (2009) Selective fluorescence labeling of lipids in living cells. Angew. Chem. Int. Ed. Engl. 48, 1498–1500.
(6) McNenay, P. J., Parker, C. G., Zhang, A. X., and Spiegel, D. A. (2012) Antibody-recruiting molecules: An emerging paradigm for engaging immune function in treating human disease. ACS Chem. Biol. 7, 1139–1151.
(7) Molbaj, M., Cramer, S., Brischwein, K., Rau, D., Sriskandarajah, M., Hoffmann, P., Kufer, P., Hofmeister, R., and Baeruele, P. A. (2007) CD19-CD3 bispecific antibody of the BiTE class is far superior to tandem diabody with respect to redirected tumor cell lysis. Mol. Immunol. 44, 1935–1943.
(8) Baeruele, P. A., and Reinhardt, C. (2009) Bispecific T-cell engaging antibodies for cancer therapy. Cancer Res. 69, 4941–4944.
(9) Parker, C. G., Domaalo, R. A., Anderson, K. S., and Spiegel, D. A. (2009) An antibody-recruiting small molecule that targets HIV gp120. J. Am. Chem. Soc. 131, 16392–16394.
(10) Mack, M., Riethmüller, G., and Kufer, P. (1995) A small bispecific antibody construct expressed as a functional single-chain molecule with high tumor cell cytotoxicity. Proc. Natl. Acad. Sci. U.S.A. 92, 7021–7025.
(11) Sedaelin, M., Brentjens, R., and Rivière, I. (2009) The promise and potential pitfalls of chimeric antigen receptors. Curr. Opin. Immunol. 21, 215–223.
(12) Sedaelin, M., Brentjens, R., and Rivière, I. (2013) The basic principles of chimeric antigen receptor design. Cancer Discovery 3, 388–398.
(13) Grupp, S. A., Kalos, M., Barrett, D., Aplenc, R., Porter, D. L., Rheingold, S., Teachey, D. T., Chew, A., Hauck, B., Wright, J. F., Milone, M. C., Levine, B. L., and June, C. H. (2013) Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N. Engl. J. Med. 368, 1509–1518.

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Morillon, E., Sorensen, R., Forster, A., Fraser, P., Cohen, J. I., de Saint Basile, G., Alexander, I., Wintergerst, U., Frebourg, T., Aurias, A., Stoppa-Lyonnet, D., Romana, S., Radford-Weiss, I., Gross, F., Valensi, F., Delabesse, E., Macintyre, E., Sigaux, F., Soulier, J., Leiva, L. E., Wissler, M., Prinz, C., Rabbitts, T. H., Le Deist, F., Fischer, A., and Cavazzana-Calvo, M. (2003) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 302, 415–419.

(15) Tomita, U., Yamaguchi, S., Maeda, Y., Chujo, K., Minamihata, K., and Nagamune, T. (2013) Protein cell-surface display through in situ enzymatic modification of proteins with a poly(ethylene glycol)-lipid. Biotechnol. Bioeng. 110, 2785–2789.

(16) Tatsumi, K., Ohashi, K., Teramura, Y., Utoh, R., Kanegae, K., Watanabe, N., Mukobata, S., Nakayama, M., IWata, H., and Okano, T. (2012) The non-invasive cell surface modification of hepatocytes with PEG-lipid derivatives. Biomaterials 33, 821–828.

(17) Antos, J. M., Miller, G. M., Grotenbreg, G. M., and Ploegh, H. L. (2008) Lipid modification of proteins through sortase-catalyzed transpeptidation. J. Am. Chem. Soc. 130, 16338–16343.

(18) Ton-That, H., Liu, G., Mazmanian, S. K., Faull, K. F., and Schneewind, O. (1999) Purification and characterization of sortase, the transpeptidase that cleaves surface proteins of Staphylococcus aureus at the LPXTG motif. Proc. Natl. Acad. Sci. U.S.A. 96, 12424–12429.

(19) Navare, W. W., and Schneewind, O. (1999) Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. Microbiol. Mol. Biol. Rev. 63, 174–229.

(20) Guimaraes, C. P., Witte, M. D., Theile, C. S., Bozkurt, G., Kundrat, L., Blom, A. E. M., and Ploegh, H. L. (2013) Site-specific C-terminal and internal loop labeling of proteins using sortase-mediated reactions. Nat. Protoc. 8, 1787–1799.

(21) Tsukiji, S., and Nagamune, T. (2009) Sortase-mediated ligation: A gift from Gram-positive bacteria to protein engineering. ChemBioChem 10, 787–798.

(22) Popp, M. W., Antos, J. M., Grotenbreg, G. M., Spooner, E., and Ploegh, H. L. (2007) Sortaging: A versatile method for protein labeling. Nat. Chem. Biol. 3, 707–708.

(23) Aulabaugh, A., Ding, W., Kapoor, B., Tabei, K., Alksne, L., Dushin, R., Zatz, T., Ellestad, G., and Huang, X. (2007) Development of an HPLC assay for Staphylococcus aureus sortase: Evidence for the formation of the kinetically competent acyl enzyme intermediate. Anal. Biochem. 360, 14–22.

(24) Chen, L., Dorr, B. M., and Liu, D. R. (2011) A general strategy for the evolution of bond-forming enzymes using yeast display. Proc. Natl. Acad. Sci. U.S.A. 108, 11399–11404.

(25) Popp, M. W.-L., Karssemeijer, R. A., and Ploegh, H. L. (2012) Chemoenzymatic site-specific labeling of influenza glycoproteins as a tool to observe virus budding in real time. PLoS Pathog. 8, e1002604.

(26) Shi, J., Kundrat, L., Pishesha, N., Bilate, A., Theile, C., Maruyama, T., Dougan, S. K., Ploegh, H. L., and Lodish, H. F. (2014) Engineered red blood cells as carriers for systemic delivery of a wide array of functional probes. Proc. Natl. Acad. Sci. U.S.A. 111, 10131–10136.

(27) Ts, H. T., Prabhu, S., Leitner, E., Jia, F., von Elverfeldt, D., Jackson, K. F., Hant, T., Nair, A. K. N., Pearce, H., von zur Muhlen, C., Wang, X., Peter, K., and Hagemeyer, C. E. (2011) Enzymatic single-chain antibody tagging: A universal approach to targeted molecular imaging and cell homing in cardiovascular disease. Circ. Res. 109, 365–373.

(28) van Werven, F. J., and Timmers, H. T. M. (2006) The use of biotin tagging in Saccharomyces cerevisiae improves the sensitivity of chromatin immunoprecipitation. Nucleic Acids Res. 34, e33.

(29) Brewster, N. K., Val, D. L., Walker, M. E., and Wallace, J. C. (1994) Regulation of pyruvate carboxylase isozyme (PYC1, PYC2) gene expression in Saccharomyces cerevisiae during fermentative and non-fermentative growth. Arch. Biochem. Biophys. 311, 62–71.

(30) Hoja, U., Marhol, S., Hofmann, J., Stegner, S., Schulz, R., Meier, S., Greiner, E., and Schweizer, E. (2004) Identification of the tRNA-binding protein Arc1p as a novel target of in vivo biotinylation in Saccharomyces cerevisiae. J. Biol. Chem. 279, 42445–42452.

(31) Hasslacher, M., Ivesa, A. S., Paltauf, F., and Kohlwein, S. D. (1993) Acetyl-CoA carboxylase from yeast is an essential enzyme and is regulated by factors that control phospholipid metabolism. J. Biol. Chem. 268, 10946–10952.

(32) Kirchhofer, B., Wenzel, J., Schmidthals, K., Frauser, C., Cui, S., Karcher, A., Pellis, M., Muyldermans, S., Casas-Delucchi, C. S., Cardoso, M. C., Leonhardt, H., Hopfner, K.-P., and Rothbauer, U. (2010) Modulation of protein properties in living cells using nanobodies. Nat. Struct. Mol. Biol. 17, 133–138.

(33) Witte, M. D., Cragnolini, J. J., Dougan, S. K., Yoder, N. C., Popp, M. W., and Ploegh, H. L. (2012) Preparation of unnatural N-to-N and C-to-C protein fusions. Proc. Natl. Acad. Sci. U.S.A. 109, 11993–11998.

(34) Håkansson, S., Morisaki, H., Heuser, J., and Sibbey, L. D. (1999) Time-lapse video microscopy of gliding motility in Toxoplasma gondii reveals a novel, biphasic mechanism of cell locomotion. Mol. Biol. Cell 10, 3539–3547.

(35) Massa, P. E., Panici, A., Monegal, A., de Marco, A., and Rescalino, M. (2013) Salmonella engineered to express CD20-targeting antibodies and a drug-converting enzyme can eradicate human lymphomas. Blood 122, 705–714.

(36) Dang, L. H., Bettegowda, C., Huso, D. L., Kinzler, K. W., and Vogelstein, B. (2001) Combination bacteriolytic therapy for the treatment of experimental tumors. Proc. Natl. Acad. Sci. U.S.A. 98, 15155–15160.

(37) Hogquist, K. A., Jameson, S. C., Heath, W. R., Howard, J. L., Bevan, M. J., and Carbene, F. R. (1994) T cell receptor antagonist peptides induce positive selection. Cell 76, 17–27.

(38) Dobrowolski, J. M., Carruthers, V. B., and Sibbey, L. D. (1997) Participation of myosin in gliding motility and host cell invasion by Toxoplasma gondii. Mol. Microbiol. 26, 163–173.

(39) Hirakawa, H., Ishikawa, S., and Nagamune, T. (2012) Design of Ca2+ independent Staphylococcus aureus sortase A mutants. Biotechnol. Bioeng. 109, 2955–2961.