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Development of a Chemoenzymatic-like and Photoswitchable Method for the High-Throughput creation of Protein Microarrays. Application to the Analysis of the Protein/Protein Interactions Involved in the YOP Virulon from Yersinia pestis.

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Development of a Chemoenzymatic-like and Photoswitchable Method for the High-Throughput creation of Protein Microarrays. Application to the Analysis of the Protein/Protein Interactions Involved in the YOP Virulon from *Yersinia pestis*.

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ABSTRACT

Protein arrays are ideal tools for the rapid analysis of whole proteomes as well as for the development of reliable and cheap biosensors. The objective of this proposal is to develop a new ligand assisted ligation method based in the naturally occurring protein trans-splicing process. This method has been used for the generation of spatially addressable arrays of multiple protein components by standard micro-lithographic techniques. Key to our approach is the use of the protein trans-splicing process. This naturally occurring process allows the development of a truly generic and highly efficient method for the covalent attachment of proteins through its C-terminus to any solid support. This technology has been used for the creation of protein chips containing several virulence factors from the human pathogen *Y. pestis*.

Introduction

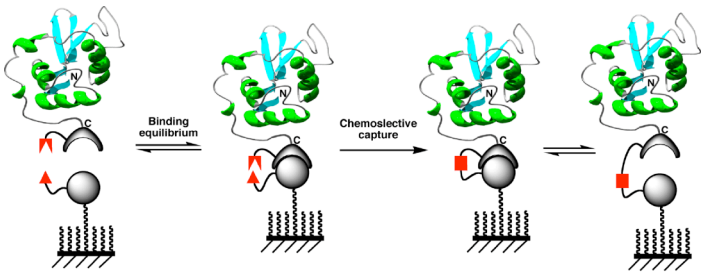
Many experimental techniques in biology and biophysics, and applications in diagnosis and drug discovery, require proteins immobilized on solid substrates.[1-3] In fact, the concept of arrays of proteins attached to a solid support has attracted increasing attention over the last three years due to the sequencing of several genomes, including the human genome. When a genome has been deciphered, the daunting task of determining the function of each protein encoded in the genome still remains. Protein arrays can be used easily for such analysis in a parallel fashion.[2,4] Another powerful application employs ordered nanometric arrays of proteins as nucleation templates for protein crystallization or for structural studies. Recent advances in nanoprinting techniques have allowed the creation of sub-micrometer arrays of proteins.[5,6] All these applications demonstrate the use of protein arrays and also highlight the need for methods able to attach proteins in a well defined and ordered way onto a solid supports.

Various methods are available for attaching proteins to solid surfaces. Most rely on non-specific adsorption,[6,7] or on the reaction of chemical groups within proteins (mainly, amino and carboxylic acid groups) with surfaces containing complementary reactive groups.[8,9] In both cases the protein is attached to the surface in random orientations. The use of recombinant affinity tags addresses the orientation issue. However, in most cases the interactions of the tags are reversible and therefore not stable over the course of subsequent assays or require large mediator proteins.[10,11] Methods for the chemoselective attachment of proteins to surfaces has been also developed recently by our group.[12-15] These methods rely in the introduction of two unique and mutually reactive groups on the protein and the support surface. The reaction between these two groups usually gives rise to the selective attachment of the protein to the surface with total control over the orientation.[14,15] However, these methods, although highly selective, rely on uncatalyzed pseudo-bimolecular reactions with little or not activation at all. This lack of activation means that the efficiency of these bimolecular-like reactions depends strongly on the concentration of the reagents. A way to overcome this intrinsic entropic barrier and make attachment reactions even more efficient and selective, even under high dilution conditions, is through the use of a highly selective molecular recognition event to bring together the two reactive species. This event increases dramatically the local effective concentration of both reacting species thus accelerating the corresponding attachment reaction even under unfavorable conditions (i.e. low concentration and even in the presence of other proteins).

The objective of this project has been to develop a new ligand assisted ligation method based in the naturally occurring protein trans-splicing process.[16-19] This methodology has allowed the development of a truly generic and highly efficient method for the covalent attachment of proteins through its C-terminus to any solid support. This new approach is totally traceless (i.e. the protein of interest will be directly attached to the surface without the requirement of large protein mediators/linkers) and it can be used for the generation of spatially addressable arrays of multiple protein components by using standard DNA-array or photo-lithographic techniques. Finally, this technology is also being used for the production of protein arrays some of the key protein components involved in the Type III Secretion System from the pathogenic bacterium *Yersinia pestis*, an enteric pathogen that is the causative agent of bubonic and pneumonic plague. Proteins from *Y. pestis* type III secretion system, include cytotoxins and effectors (Yops and Lcr-related proteins and usually referred as YOP proteins) that are secreted into eukaryotic cells and inhibit bacterial phagocytosis along with innate immunity and may be ideal targets for mitigating pathogenicity.[20,21]

Background

Most of the available methods for the chemoselective attachment of proteins to surfaces are based on uncatalyzed bimolecular-like reactions with little or not entropic activation at all. This lack of entropic activation means that the efficiency of these bimolecular-like reactions will depend strongly on the concentration of the reagents (i.e. on the concentration of the protein to be attached to the corresponding surface). This is basically due to the loss of entropy associated by the two reacting groups having to be brought together in the right orientation for the reaction to happen. A way to overcome this intrinsic entropic barrier and make ligation reactions more efficient even under high dilution conditions is using specific non-covalent interactions for bringing both reactive groups in close proximity (Scheme 1). Under these conditions the rate of bimolecular protein chemical ligation reactions should be very efficient even



Scheme 1. *Principle for the attachment of a protein to a surface by using an entropically activated chemoselective reaction.*

under high dilution conditions. This effect was first reported by Sheppard and co-workers,[22] who noticed that pancreatic trypsin inhibitor fragments obtained by CNBr cleavage were able to spontaneously religate forming the original native peptide bond between them. The same effect was also observed for cytochrome C.[23] In both cases, the two protein fragments produced after the CNBr cleavage, were able to cooperatively refold thus bringing the homoserine lactone at the C-terminus of one of the fragments in close proximity of the α-amino group of the other fragment. The high local concentration effect of these groups and the mild activation of the homoserine lactone functionality resulted in spontaneous amide bond formation. More recently, Mrksich and co-workers[24] have used this same principle for the selective attachment of protein onto surfaces with total control over the orientation. In their approach, they used the protein calmodulin fused with the enzyme cutinase as a capture protein. Cutinase is a 22 kDa serine esterase that is able to form a site-specific covalent adducts with chorophosphonate ligands.[25] In this case the ligand (a chlorophosphonate group) mimics the tetrahedral transition state of an ester hydrolysis. When it binds specifically to the active site of the enzyme, the hydroxyl group of the catalytic serine residue reacts covalently with the chlorophosphonate to yield a stable covalent adduct that is resistant to hydrolysis This results in the covalent and selective attachment of the protein to the surface through the capture protein that acts like a linker. The attachment is selective enough to allow the selective attachment of cutinase fusion protein from crude *E. coli* periplasmic lysates, thus demonstrating that the protein to be immobilized does not need to be purified before the immobilization step.

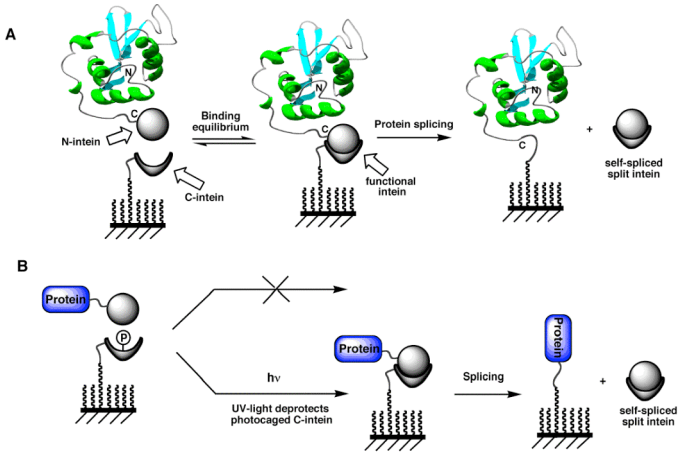


Figure 1. *Attaching proteins to solid surfaces by using a protein trans-splicing technique.* ***A.*** *Principle of the protein trans-splicing approach for the immobilization of proteins. The C-intein fragment is attached to the surface and the N-intein fragment is fused to the C-terminus of the protein to be attached. When this fusion protein is exposed to C-intein-containing surface, the two intein fragments associate yielding a fully operational intein domain that then splices out attaching at the same time the protein to the surface.* ***B.*** *The attachment of proteins using this technique can be easily controlled by photo-caging the C-intein fragment. Under this conditions the intein complex can not be formed and consequently the protein is not attached. Only when the photo-labile group is removed by action of UV light the C-intein can yield the active*

Selective attachment of proteins by protein trans-splicing. One of the limitations of the previous approach, however, is that the linker between the protein of interest and the surface is always another protein. The presence of such a big linker could potentially give rise to some problems, specially in those applications where the attached protein will be involved in studying protein/protein interactions with complex protein mixtures,[2,4] mainly due to potential cross-reactivity issues.

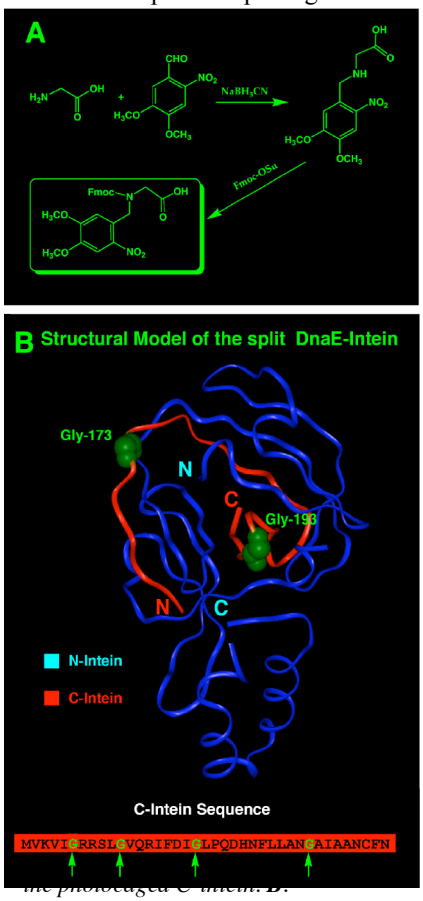
In order to solve this problem, our group has developed a new method for the chemoenzymatic-like attachment of proteins to surfaces based on the protein trans-splicing process (Fig. 1).[26] This process is similar to the protein splicing with the only difference that in this case the intein self-processing domain is split in two fragments (called N-intein and C-intein, respectively).[16,27-30]

These two intein fragments alone are inactive, however, when they are put together under the appropriate conditions they bind specifically each other yielding a totally functional splicing domain, which it splices itself at the same that ligates both extein sequences. In our approach, one of the fragments (C-intein) is covalently attached to the surface through a small peptide-linker meanwhile the other fragment (N-intein) is fused to the C-terminus of the protein to be

attached to the surface. The interaction between the two intein fragments allows the protein of interest to be immobilized onto the surface through protein trans-splicing (see Fig. 1).

Technical Plan

Selective protein attachment for high throughput protein chip production. Key to this approach is the use of the highly specific and effective protein trans-splicing process. This naturally occurring process is similar to the protein splicing with the only difference that in this case the intein self-processing domain is split in two fragments (called N-intein and C-intein, respectively). In our approach, one of the fragments (C-intein) is covalently immobilized onto the surface through a small peptide-linker (see Fig. 3) meanwhile the other fragment (N-intein) is fused to the C-terminus of the protein to be attached to surface. The immobilization of the protein through protein trans-splicing is highly specific thus eliminating the need for the purification and/or reconcentration of the proteins prior to the immobilization step. More importantly, once the protein is immobilized to the surface, both intein fragments are spliced out into solution, providing a completely traceless method of attachment.



Structural model of the split DnaE-intein showing the Gly residues that will be photocaged in order to prevent the association of the C-intein and N-intein fragments.

Another key aspect of our approach is the potential to create spatial addressable protein arrays with multiple protein components using standard pholithographic techniques. This can be easily accomplished by creating a C-intein fragment where some of the key residues for the interaction with the N-intein are caged with a protecting group removable by UV-light (i.e. photocaged, see Fig. 2). This blocked C-intein fragment will be unable to assemble with the N-intein fused protein thus preventing protein trans-splicing to occur. The deprotection of these groups by the action of UV-light, however, allows the two intein fragments to assemble thus allowing the attachment of the corresponding protein to the surface through protein splicing (see Fig. 1B). Finally, the high efficiency and selectivity of this new protein immobilization approach allows to be easily interfaced with high throughput protein expression systems such as coupled in vitro transcription/translation systems.

High throughput protein cloning, expression for proteins of interest. YOP and other proteins will be cloned using high-throughput cloning techniques and expressed in *E. coli.*[31] The use of coupled *in vitro* transcription/translation systems will also be used for high throughput screening and production of functional YOP proteins.[26]

High throughput specific labeling of YOP proteins. YOP proteins will be specifically labeled with orthogonal Alexa or similar dyes (up to 4 different fluorescent dyes will be used for multiplexed studies) at their C- or N-termini. This allows the screening of the corresponding protein microarrays with up to 4 different proteins at the same time, thus allowing the study of potential multicomponent protein complexes. Selective labeling will be accomplished by using Expressed Protein Ligation (EPL).[32,33] Briefly, this novel methodology allows the chemosective ligation at the C- or N-termini of the protein of interest to a small synthetic peptide that is specifically labeled with a particular fluorescent dye.

Results

Site-specific immobilization of proteins through protein trans-splicing. We have developed a new traceless capture ligand approach for the selective immobilization of proteins through their C-termini to a modified glass surface (Figure 1). Our approach is based on the use of protein trans-splicing [16,34], which is a naturally occurring process similar to protein splicing [17,19,35,36] with the difference that the intein self-processing domain is split in two fragments (called N-intein and C-intein, respectively). These two intein fragments are inactive individually. However, they can bind each other with high specificity under appropriate conditions to form a functional protein splicing domain. In our approach, the C-intein fragment is covalently immobilized onto a glass surface through a PEGylated-peptide linker while the N-intein

fragment is fused to the C-terminus of the protein to be attached to surface. When both intein fragments interact, they form an active intein domain, which ligates the protein of interest to the surface at the same time the split intein is spliced out into solution (Figure 1).

Key to our approach is the use of the naturally split DnaE intein from *Synechocystis sp*. PCC6803.[16] The C- and N-intein fragments of the DnaE intein are able to self-assemble spontaneously (K_d = 0.1-0.2 μM)[26] not requiring any refolding step. The DnaE intein-mediated trans-splicing reaction is also very efficient under physiological-like conditions ($\tau_{1/2} \approx 4$ h and trans-splicing yields ranging from 85% to almost quantitative)[26]. The effect of pH and temperature on trans-splicing mediated by the naturally split DnaE intein from *Synechocystis sp*. PCC6803 has been also extensively studied.[18] The optimal pH for trans-splicing is around 7.0, however, the trans-splicing reaction was also relatively efficient at pH values ranging from 5 to 8. To test the suitability of protein trans-splicing for selective protein immobilization onto solid surfaces, we used two model proteins, Maltose Binding Protein (MBP) and Enhanced Green Fluorescent Protein (EGFP). Both proteins were modified at the DNA level to append the DnaE N-intein fragment (I_N, residues 1-123 of the DnaE intein) at their C-termini. The two fusion proteins (MBP-I_N and EGFP-I_N) were readily expressed in *E. coli* and purified by affinity chromatography. In order to enable the site-specific attachment of the I_N fusion proteins onto a glass surface, an amine-functionalized glass slide was first treated with maleimidopropionic acid N-hydroxysuccinimide ester (MPS) and then reacted with a mixture of PEGylated thiol linkers 1 and 2, in a molar ratio of 3:97, respectively (Figure 3). Both linkers contain a PEG moiety, which prevents non-specific interactions and also acts as a hydrophilic spacer, minimizing any detrimental interaction between the attached protein and the glass surface.[13]

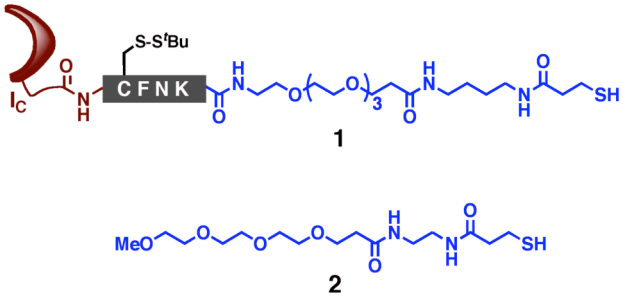


Figure 3. *Structures of linkers **1** and **2** used for the derivatization of glass slides to be used in the immobilization of proteins through protein trans-splicing.*

Linker 1 contains the DnaE C-intein fragment (I_C, residues 124-159 of the DnaE intein) followed by the corresponding C-extein sequence CFNK. The Cys residue in the C-extein sequence was protected with a S-^{t}Bu protecting group. This was required to ensure that the I_C-containing linker was selectively immobilized through its PEGylated terminal thiol onto the maleimido-coated glass surface (Figure 3). Linker 2 was used as diluent to control the density of reactive sites on the modified glass surface. When the derivatization was complete, the S-^{t}Bu protecting group on the Cys residue was removed with 50% β-mercaptoethanol in dimethylformamide (DMF) for 2 h. The surface was washed and immediately used for spotting. Dried I_C-coated glass slides could be stored for several months at -20°C before being used. In order to increase the stability of the I_C polypeptide, however, the dried I_C-coated glass should be stored with the C-extein Cys residue protected.

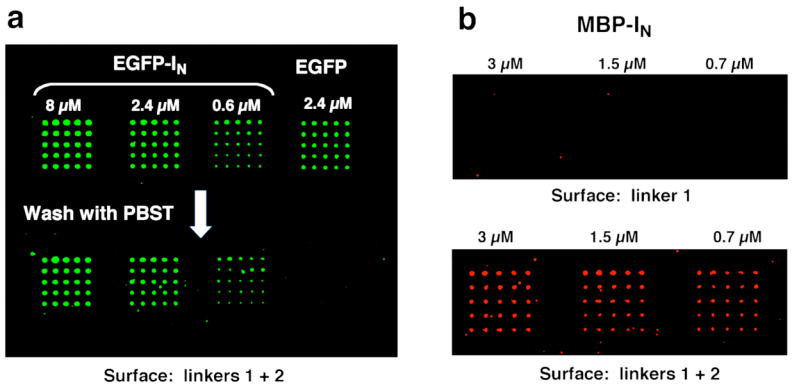


Figure 4. *Selective attachment of EGFP-I_N (green) and MBP-I_N (red) onto derivatized glass slides. (a) Epifluorescence image of an I_C-coated glass slide after the spotting of different concentrations of EGFP-I_N and EGFP (top) and after buffer washes (bottom). (b) Different concentrations of MBP-I_N were spotted onto a PEG- (top) and I_C-coated (bottom) glass slides. After washing, the immobilized MBP was detected by immunofluorescence.*

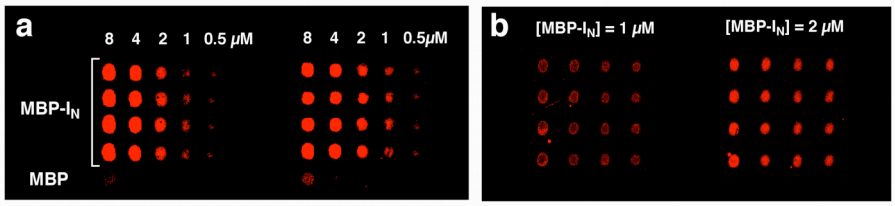


Figure 5. *Selective immobilization of MBP-I_N from complex mixtures. (a) Soluble cellular fraction of E. coli cells overexpressing MBP-I_N. (b) MBP-I_N expressed in vitro using a cell-free system. Protein concentrations in the cell lysate and IVT crude reaction were estimated by Western Blotting. In both cases, MBP was detected by immunofluorescence after washing. The level of non-specific background can be seen at the bottom of the slide when MBP with no DnaE N-intein was spotted.*

Different concentrations of pure EGFP-I_N and MBP-I_N fusion proteins were spotted onto I_C-coated glass slides (Figure 4). As a control, a solution of EGFP with no I_N fragment was spotted in the same slide (Figure 4a). Also, a solution of MBP-I_N was spotted onto a glass slide derivatized with only the non-functional linker 2 (Figure 4b). The trans-splicing reaction was incubated for 16 h in a humidified chamber at 37°C. As shown in Figure 4, only specific immobilization of the proteins containing the I_N polypeptide to the I_C-containing glass surfaces was observed. No fluorescent signal was detected from the control protein EGFP, lacking the I_N polypeptide, after washing (Figure 4a). Also, the MBP binding was minimal when the trans-splicing active MBP-I_N fusion protein was spotted onto a control glass slide coated with the non-functional linker 2 (Figure 4b, top panel). The trans-splicing mediated attachment of EGFP and MBP in both cases was efficient in a range of concentrations (Figure 4). Also, it is interesting to note that the attached EGFP retained its characteristic green fluorescence, indicating that its tertiary structure was unaffected by the attachment to the PEGylated glass surface. The covalent nature of the new bond formed between the C-terminus of the protein and the modified surface was confirmed by washing a glass slide

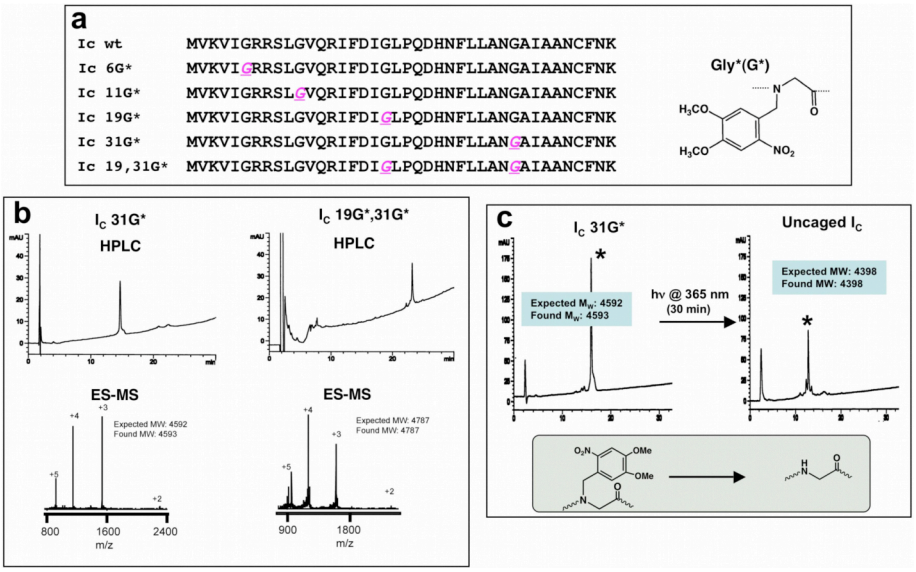


Figure 6. *Expression and characterization of the different photocaged versions of the DnaE C-intein polypeptide. (a) Sequences of the different photocaged I_C polypeptides synthesized in this project. Chemical structure of the protecting group used to photocaged the Gly residue (b) Characterization of purified products by analytical HPLC and ES-MS. (c) HPLC and ES-MS analysis of the photodeprotection reaction for I_C 31G* performed with UV light at 365 nm for 30 min.*

containing immobilized MBP with a 2% SDS solution (SDS solutions above the critical micelle concentration have been previously used to desorb non-specifically adsorbed proteins[10] as well as to disrupt specific protein/protein interactions[37]). Once the stringent wash was complete and the glass slide was re-stained to detect the remaining attached MBP, no significant loss of fluorescence was detected. This result confirms that the immobilization of the proteins to the surface proceeds mostly through the formation of a stable covalent bond and not through a transient affinity interaction.

The ability to selectively immobilize I_N-containing fusion proteins from complex mixtures through protein trans-splicing has been also explored. Hence, MPB and MBP-I_N fusion proteins were overexpressed in *E. coli,* and the corresponding soluble cellular fractions were spotted onto I_C-coated glass slides as described above. As before, the immobilization of the MBP protein containing the I_C polypeptide was extremely selective with minimal background (Figure 5a). Encouraged by these results, we investigated the possibility of interfacing this new immobilization technique with cell-free expression systems. Thus, the MBP-I_N was expressed using an *E. coli*-based *in vitro* transcription/translation (IVT) system. The crude IVT reaction was spotted without further treatment, and it was processed as described above. As before, the MPB protein was selectively attached to the I_C-coated glass surface (Figure 5b). In both cases, the amount of immobilized protein was shown to be similar.

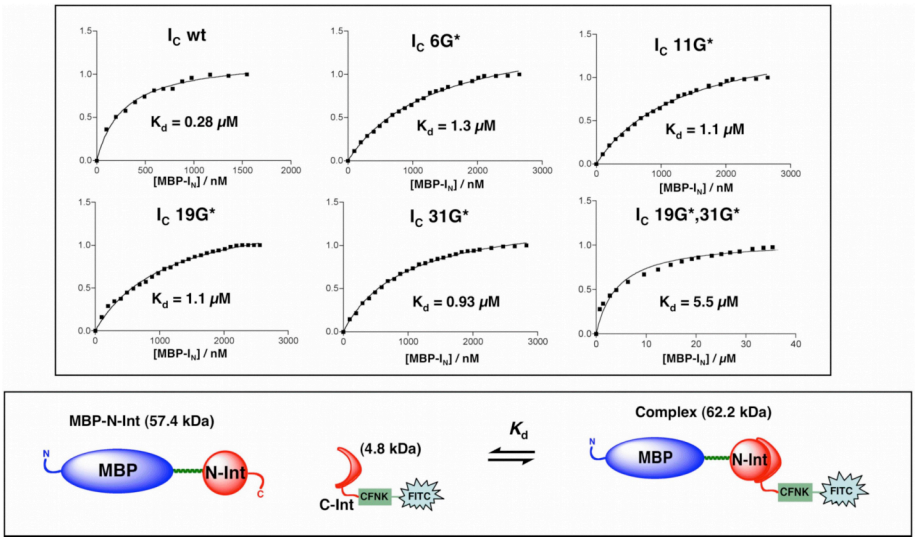


Figure 7. *Isotherm binding curves for the interaction between MBP-I_N and the different photocaged I_C polypeptides. Shown is the change in fluorescence polarization of fluoresceine-labeled I_C as a function of added MBP-I_N fusion protein.*

Photomodulation of protein trans-splicing. Several photocaged DnaE C-intein polypeptides were successfully synthesized using optimized Fmoc-based Solid-Phase Peptide Synthesis (Figure 6). After purification by preparative HPLC, the purified photocaged peptides were characterized by analytical HPLC and mass spectrometry (Figure 6b). Removal of the o-nitrobenzyl backbone protecting group was efficiently accomplished by irradiating for 30 min with UV light at 365 mn a solution of the corresponding photocaged peptide (Figure 6c). The binding affinities of the different photocaged C-intein polypeptides for the unmodified N-intein polypeptide were also studied by fluorescence anisotropy. As shown in Figure 7, the dissociation constant for the C-intein polypeptides containing only one photocaged Gly residue was around six times weaker than the wild type C-intein polypeptide. The polypeptide containing two photocaged Gly residues (I_C 19G*,31G*), on the other hand, showed an affinity constant for the N-intein significantly much weaker, around 37 times lower than the observed for the wild-type I_C. The trans-splicing activity of all the photocaged C-intein polypeptides was also tested using MBP-I_N. All the single photocaged I_C polypeptides showed significant decreases in their ability to promote trans-splicing as shown in Figure 8a. Polypeptide I_C 31G* was by far the more efficient in preventing trans-splicing, showing only a 10% of the trans-splicing activity of the I_C wt polypeptide (Figure 8a) under the same reaction conditions. I_C 19G* and 6G* both showed ≈30% of the I_C wt trans-splicing activity. I_C 11G* was the less efficient of the group preventing trans-splicing, showing around 60% of the I_C wt activity. In contrast, the polypeptide I_C

19G*,31G*, which contains two photocaged Gly residues, was able to completely prevent trans-splicing activity (Figure 7b).

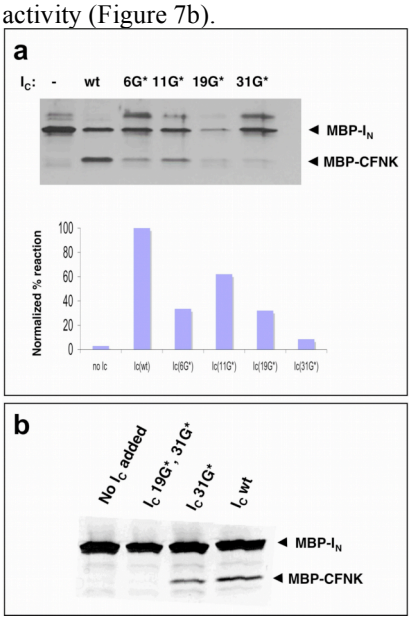


Figure 8. *Trans-splicing activity of single (a) and double (b) photocaged DnaE I_C polypeptides with MBP-I_N fusion protein. In all the cases the trans-splicing reactions were carried out under the same conditions. Reactions were analyzed by SDS-PAGE. The trans-spliced product MBP-CFNK was isolated by analytical HPLC and characterized by mass spectrometry (data not shown).*

High throughput protein cloning, expression for proteins of interest. During the duration of this project several Yop-DnaE I_N fusion proteins have been cloned and expressed in *E. coli* and cell-free systems (see Figure 9). Yop proteins from Co92 strain were amplified by PCR using genomic DNA (kindly provided by Matthew Coleman/Emilio Garcia, CMLS). The DNA fragments were purified and inserted between the *Nde* I and *Bam*H I restriction sites of either a DnaE-I_N-pIVEX2.3 plasmid (Roche Diagnostics) or DnaE-I_N-pET28a plasmid. *In vivo* and *in vitro* expression and characterizations was carried out as previously described.[26]

Significance and potential results.

The ability of attaching proteins to a solid support in an ordered fashion is expected to play a critical role in several key applications in the field of biology and biophysics. For example, the creation of functional protein microarrays is critical for the progress in proteomics research. Like DNA chips, protein chips allow the analysis of thousand of proteins simultaneously for the rapid discovery of new drug targets.

We have started a collaborative effort to use this newly developed technology to create protein arrays capable of characterizing known and unknown protein interactions involved in the Type III Secretion System from the pathogenic bacterium *Y.pestis*. This human pathogen is the etiologic agent of bubonic and pneumonic plague and it has been recognized as one of the most devastating, epidemic-causing bacteria experienced by mankind. Although plague is not a public health problem in most parts of the world, its potential for contagion, the lack of an effective vaccine, and the recent emergence of multiple antibiotic-resistant strains place this organism squarely at the top of the United States' select agent list as a potential candidate for bioterrorism use. For these reasons, the development of highly effective anti-plague treatments, particularly to combat strains of *Y. pestis* resistant to traditional drugs is an immediate public health priority.

The use of Yop-based protein microarrays will provide an invaluable tool to study and establish new protein-protein interactions under different environmental conditions as potential targets to mitigate pathogenicity. Therefore, these Yop-based protein microarrays could also be used for rapid screening of chemically synthesized libraries to identify potential inhibitors of key protein interactions for virulence in *Y. pestis*.

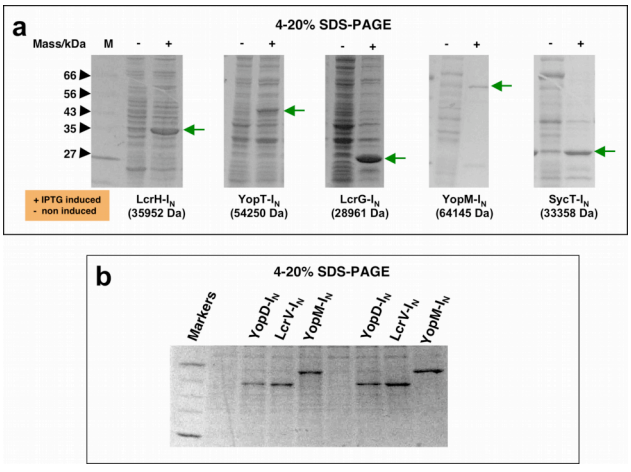


Figure 9. *Expression of several Yop-IN fusion protein in E. coli (a) or in cell-free systems (b). The target proteins are indicated by an arrow.*

Exit Plan

This whole project aligns extremely well with the National Institute of Allergy and Infectious Diseases (NIAID) and Department of Homeland Security (DHS) strategic plan for

biodefense research and the LLNL responsibilities to homeland security. We anticipate that on the bases of results obtained from this proposal, we would be in a very competitive position to apply for external funding from NIAID and DHS.

Summary

In summary, we have demonstrated a new strategy for the site-specific immobilization of proteins onto solid supports by protein trans-splicing. The immobilization of the protein is highly specific and efficient thus eliminating the need for the purification and/or reconcentration of the proteins prior to the immobilization step. More importantly, once the protein is immobilized to the surface, both intein fragments are spliced out into solution, providing a completely traceless method of attachment. Finally, this methodology can be easily interfaced with cell-free protein expression systems, allowing for rapid access to the high-throughput production of protein chips.

Peer-reviewed publications generated during this project

1) Y.-H. Woo and J. A. Camarero (2006) Interfacing 'Hard' and 'Soft' matter with exquisite chemical control, *Curr. Nanoscience* **2**, 93-103.
2) Y. Kwon, M. A. Coleman and J. A. Camarero (2006) Selective immobilization of proteins onto solid support through split-intein mediated protein trans-splicing, *Angew. Chem. Int. Ed.*, **45**,1726-1729 (This manuscript has been highlighted in the Spotlight section of ACS Chemical Biology 1(3), in press).
3) J. A. Camarero (2006) New methods for the selective attachment of proteins to surfaces*, Biophys. Rev. Lett.* **1**, 1-28.
4) Y. Kwon, M. A. Coleman and J. A. Camarero (2006) New tools for the site-specific attachment of proteins to surfaces in *Understanding Biology using Peptides* (Ed.; Blondelle, S. E.), Springer, New York, pp. 780-782.
4) J. A. Camarero (2005) Editorial Hot Topic: Polypeptide Chemical Ligation Tools in Protein Engineering, *Protein Pept. Lett.* **12**, 721-722.
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Presentations in scientific conferences

1) Invited Keynote speaker at the Modern Solid Phase Peptide Synthesis & Its Applications, 7th International Australian Symposium, October 17-19, 2007, Port Douglas, Australia.
2) Invited oral presentation at the International Conference on Materials for Advanced Technologies, July 1-6, 2007, Singapore.
3) Invited oral presentation at the CHI PepTalk 2007, 6th Annual Protein Arrays Meeting, January, 11-12, 2007, San Diego, CA.
4) Poster presentation at the 20th Symposium of the Protein Society, August 5-9, 2006, San Diego, CA.
5) Poster presentation at the 232nd American Chemical Society National Meeting & Exposition, September 10-14, 2006, San Francisco, CA.
6) Oral presentation at the 19th American Peptide Symposium, June 18-23, 2005, San Diego, CA.

Patents and records of invention:

1) A new chemoenzymatic-like and photoswitchable method for the ordered attachment of proteins to surfaces, J. A. Camarero, Y. Kwon, J. J. de Yoreo, invention case No. IL-11195 (Published US Patent #20050095651).
2) Preparation of peptide p-nitroanilides using an aryl hydrazine solid support, J. A. Camarero, A. R. Mitchell, Y. Kwon, invention case No. IL-11413 (DOE case #S-105,612).

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