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Directed surface attachment of nanomaterials via coiled-coil-driven self-assembly

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Abstract

Numerous nanoscale devices and materials have been fabricated in recent years using a variety of biological scaffolds. However, the interfacing of these devices and materials into existing circuits and ordered arrays has proved problematic. Here, we describe a simple solution to this problem using self-assembly of the peptide coiled-coil heterodimer ACID:BASE to immobilize M13 bacteriophage particles to specific locations on a patterned gold surface. Surface plasmon resonance demonstrated that free ACID peptides will assemble onto a surface derivatized with BASE. We then displayed the ACID peptide on the pIX coat protein of M13 and showed that these phage particles permit formation of the coiled-coil resulting in specific surface attachment. The ACID:immobilized BASE affinities appear to be similar for free peptide and phage-displayed ACID. Finally, we fabricated two gold electrodes, separated by a 200 nm gap, coated one of them with BASE and showed that this allows localization of the M13:ACID onto the functionalized electrode.

1. Introduction

The ability of biological systems to self-assemble into complex, three-dimensional functional structures is rapidly emerging as a scalable and economic approach for the synthesis of nanoscale materials and devices. A notable example involves the M13 bacteriophage that has been used to assemble a wide range of nanomaterials and devices from simple 1D systems, e.g. nanowires, to higher order structures where multiple phage particles assemble into a specific, ordered arrangement [1, 2]. Modification of the M13 genome to encode material-specific binding peptides into phage coat proteins also enables

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the assembly of a wide range of functional materials on the surface of the M13 capsid, broadening the possible applications and functions. For example, a number of inorganic and molecular materials have been bound to the outside of the M13 capsid including: gold ions, leading to production of conducting nanowires [1]; magnetic nanoparticles and silica [3]; quantum dots [4]; and, carbon nanotubes [5, 6].

Despite these successes, the ability to position and join individual components in an organized and integrated arrangement, and to interface them with the outside world, remains a significant challenge. To date, the assembly and integration of nanoscale components synthesized in solution has been achieved typically by defining interconnects lithographically to materials dispersed randomly onto a surface. While useful for studying the fundamental characteristics of self-assembled nanomaterials, the approach is inherently serial, limiting the complexity of integrated circuits that can be assembled. An alternative approach capable of organizing nanoscale components in a scalable and highly parallel fashion has been demonstrated that uses molecular linkers, such as DNA oligonucleotides, able to bind individual nanomaterials with high selectivity and specificity and to direct their assembly into ordered arrangements. Base pairing between complementary oligonucleotides has been employed successfully to assemble nanoparticles into ordered arrays [7].

For nanoscale elements assembled from peptides and proteins, such as those templated using the M13 capsid, it would be more beneficial to use a linker based on peptides which can be expressed directly and at specific locations within the phage particle. In Nature, the assembly of proteins into macromolecular complexes is often achieved by a number of distinct protein motifs that regulate non-covalent protein–protein interactions. One of the best known of these structural motifs is the coiled-coil that is thought to be present in 3%–5% of all proteins [8]. The motif is characterized by 2–7 individual α -helical segments that wrap around one another to form a left-handed helical ‘supercoil’ [9]. Each α -helix within the coiled-coil bundle consists of a heptad repeat where the amino acid sequence is denoted **a–b–c–d–e–f–g**. Positions **a** and **d** are typically hydrophobic, e.g. Leu, Ile, Val or Ala, and interlock with hydrophobic residues at the corresponding positions (**a**’ and **d**’) of the complementary coil to form the core of the supercoil; commonly referred to as ‘knobs-into-holes’ packing [10]. Electrostatic interactions resulting from charged residues at positions **e** and **g** also contribute to supercoil stability and determine the selectivity of heterodimers over homodimers [11]. The presence of a single asparagine residue at the **a**’ position of one of the heptad repeats [12] has been shown to favour the formation of an anti-parallel coiled-coil preferentially over a parallel supercoil, i.e. in the parallel orientation both helices line up N- to N-terminus, whereas in the anti-parallel orientation the N-terminus of one helix is adjacent to the C-terminus of the other. The solvent exposed hydrophilic residues at positions **b**, **c** and **f** have only a minor influence on supercoil stability [13]. Despite the simplicity of these inter-molecular forces, coiled-coil interactions are highly specific and can have affinities in the nanomolar range [14]. Furthermore, the interactions are reversible and controllable, with the coils dissociating at the extremes of pH [2, 9, 15].

The majority of research into coiled-coil structural motifs has focused on assembly in solution [15] and stability studies of pre-assembled coiled-coils upon surfaces [16]. However, the use of coiled-coil motifs for controlled and directed assembly of nanoscale

complexes, including their integration into microscopic solid devices, has largely been neglected. Limited previous studies in this area focused on the on-surface formation of coiled-coils [17–19]. Here, we investigate the on-surface assembly of coiled-coil heterodimers and demonstrate immobilization strategies that maintain interaction specificity. Using this approach, we exploit the specificity of coiled-coil interactions to facilitate the selective integration of nanoscale components into specific locations on a patterned solid surface. Specifically, we have investigated the on-surface assembly of the anti-parallel ACID:BASE [12, 20] coiled-coil pair (figure 1). The parallel pairing of this combination has been studied extensively in solution previously [15, 21].

2. Materials and methods

2.1. Materials

Peptides were purchased from Peptide Protein Research Ltd (Fareham, Hampshire, UK). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

2.2. Creation, production and purification of M13pIXACID phage

The DNA sequence corresponding for the ACID peptide sequence was cloned into the *IX* gene of the pCGMT-1b phagemid vector (kindly provided by Dr Bin Zhou, The Scripps Research Institute, La Jolla, CA, USA) following a previously published method [22]. This system results in the ACID peptide displayed at position 1 of the pIX coat protein. A hexahis tag (HHHHHH) was also inserted at the N-terminal end of the ACID peptide (final peptide sequence displayed on pIX:

HHHHHHAQLEKELQALEKELQALEWENQALEKELQALQSGGSG). The phagemid was then transformed into DH5a *E.coli* cells (New England Biolabs, Hitchin, UK). A 1 l culture (LB medium, containing tetracycline, carbenicillin and 2% glucose) was grown for ~3 h until an OD₆₀₀ between 0.5 and 0.7 was reached. The 1 l of culture was then poured into a 6 l conical flask containing 4 l of LB medium containing tetracycline, carbenicillin, IPTG (final concentration 1 mM) and M13KO7 (final concentration 4×10^8 pfu ml⁻¹; New England Biolabs). The 5 l culture was swirled gently to mix and left to stand at room temperature for 30 min and then incubated at 30 °C, 220 rpm, for 2 h. Kanamycin was added and the 5 l culture divided into 5 × 1 l in 2 l flasks which were incubated at 30 °C, 220 rpm, overnight. × The phage were purified by adding 1/6th volume of 20% PEG, 2.5 M NaCl.

The 5–10% of the population displaying one ACID sequence on the pIX coat protein were purified using a 5 ml HisTrapTM HP column (GE Healthcare, Hatfield, UK). These are pre-packed with Ni Sepharose High Performance resin charged with Ni²⁺. An ÄKTAexplorerTM (GE healthcare) using the UNICORN software was used to control and monitor the column. The column was equilibrated with running buffer (50 mM Tris-HCl, pH 7) and the phage loaded onto the column at a flow rate of 0.5 ml min⁻¹. 3 column volumes of running buffer were used to wash the column of any phage bound non-specifically. Elution of the bound phage was done using a linear gradient of 0–400 mM imidazole. Fractions containing phage were pooled and PEG/NaCl precipitated.

2.3. Surface plasmon resonance (SPR) analysis of ACID peptide and M13pIXACID

All peptides and phage were dialysed into the running buffer (100 mM sodium phosphate, pH 7) before being used for SPR. SPR was performed using a Biacore 3000 (GE Healthcare) and carried out at room temperature. Sensor chips containing a bare gold surface (GE Healthcare) were used for experimental work. Thiolated peptides (500 RU) were immobilized using gold–thiol chemistry in running buffer. All injections were carried out at a flow rate of 10 $\mu\text{l min}^{-1}$.

2.4. Fabrication of gold electrodes and immobilization of peptides

The gold electrode array was fabricated and functionalized with specific polypeptides using electron-beam lithography and lift-off processing as previously reported [23, 24]. Each electrode was coated with the random-coil peptide BLK-C (overnight incubation in 0.5 mg ml^{-1} peptide in 100 mM sodium phosphate, pH 7), which inhibits non-specific binding of polypeptides and M13 phage particles [19]. The thiol modification introduced by the cysteine residue on BLK-C not only allows covalent and spontaneous assembly onto the gold electrodes but also provides a method for removing the masking layer selectively from each electrode through electrochemical reduction of the Au–S bond. BLK-C layer was removed from an individual electrode by cycling the electrochemical potential between 0 and -1.3 V versus Ag/AgCl five times followed by a 1 min hold at -1.3 V versus Ag/AgCl (this is to ensure complete removal of the original layer). A second potentiostat was used to hold the potential of neighbouring electrodes at -0.2 V versus Ag/AgCl during the desorption process. Having removed the molecular mask, the exposed gold electrode was functionalized by incubation in a solution of 50%:50% BASE-C:BLK-C for 5 min. This process can be repeated to functionalize each electrode within the array with different peptides.

2.5. Colour detection of ACID:BASE-C heterodimer formation

250 μl of ACID-C peptide (1 mM), in 100 mM sodium phosphate buffer (pH 7), was reduced with 5 μl of 500 mM tris(2-carboxyethyl)phosphine (TCEP) for 30 min. Biotin-maleimide (50 mM in DMSO) was added to the reduced ACID-C peptide to a final concentration of 5 mM and incubated for 4 h at room temperature. Biotinylated peptide was purified away from free biotin using a PD-10 column (GE Healthcare). Electrodes were created as above and incubated with 50 μl of biotinylated ACID-C (100 μM) for 1 h. Electrodes were washed for 60 min with TBS pH 7.5 (6 changes of buffer) and then blocked with TBS (0.5% Tween-20) for a further 60 min. Just prior to use, 6 μl of streptavidin–alkaline phosphatase (from the Promega, Madison, WI, Transcend kit) was added to 15 ml of TBS (0.5% Tween-20). The blocking buffer was poured off the electrodes and the streptavidin–alkaline phosphatase added for 50 min before being washed with TBS (2×1 min) and water (2×1 min). Western Blue Stabilized Substrate for Alkaline Phosphatase (Promega) was then incubated with the electrodes for 10 min in the dark before being gently washed with water.

2.6. Immobilization of M13pIXACID phage onto a gold electrode

Electrodes were created as above and coated with either BLK-C or 50:50 mix of BASE-C/BLK-C. Electrodes were then incubated with 1 ml of 1×10^{11} virions ml^{-1} in pH 9 100 mM sodium phosphate overnight. Electrodes were then washed thoroughly with water to remove excess phage.

3. Results and discussion

We showed previously that addition of a single cysteine residue at the C-terminus of BASE peptides, separated from the coiled-coil forming residues by a flexible linker sequence (GGGS) natively present within the pIII coat protein of M13, allows directed derivatization on thiol reactive surfaces, such as gold [19, 25]. This modified BASE peptide is herein referred to as BASE-C and we have shown that the additional C-terminal residues have little, if any, deleterious effect on the self-assembly of the ACID:BASE-C heterodimer.

To investigate the formation of the BASE-C:ACID heterodimer on a surface we used SPR. BASE-C (0.01 mg ml^{-1} in 0.1 M sodium phosphate buffer, pH 7) was immobilized covalently to the sensor chip surface via the cysteine residue by exposure of the gold sensor surface to 10 μl of solution at a flow rate of 10 $\mu\text{l min}^{-1}$, resulting in a stable SPR signal ~ 500 RU greater than the background level of the un-functionalized flow-cell. This value is an order of magnitude larger than that observed for non-specific adsorption of an equivalent cysteine-free peptide (results not shown), confirming chemoselective derivatization of BASE-C. The SPR response is equivalent to a peptide layer consisting of around 8×10^{12} molecules cm^{-2} . This compares to the density of a molecular monolayer assembled from alkane thiolates which is $\sim 4 \times 10^{14}$ molecules cm^{-2} . In order to saturate any additional covalent binding sites on BASE-C derivatized flow-cells an additional exposure to a short, cysteine-modified peptide, called BLK-C was used (figure 1). BLK-C is not predicted to adopt any defined secondary structure and showed no specific interaction with ACID (see below).

SPR sensorgrams of a titration of ACID peptide across the BASE-C functionalized surface are shown in figure 2(A). Above $\sim 10 \mu\text{g ml}^{-1}$ ACID, the SPR signal increases with increasing ACID concentration, saturating 0.5 mg ml^{-1} . Fitting a Langmuir isotherm to the plot of concentration against SPR response at 300 s, when binding was at a maximum, (figure 2(B)) yielded a dissociation constant, K_d , of $\sim 30 \mu\text{M}$ for the interaction of ACID and the BASE-C layer. This is a significantly lower affinity than previous solution phase measurements for formation of the ACID:BASE dimer, e.g. 30 nM for the parallel orientated pair [15]. This difference may reflect the steric effects of immobilization of one partner or differences in the affinity between the parallel [15] and anti-parallel (used in this study) ACID:BASE orientations. The ACID:BASE orientation is dependent upon the position of an asparagine residue within the peptide sequence [12]. Similar ACID titrations (figure S1 available at stacks.iop.org/Nano/23/495304/mmedia) were performed on immobilized layers consisting of just the BLK-C peptide or a second cysteine-modified peptide, JUN-C, which is capable of forming a specific coiled-coil with its natural FOS partner but has no affinity for ACID [2]. No binding of ACID, up to a concentration of 0.1 mg ml^{-1} , was seen, confirming the selectivity of ACID:immobilized BASE-C interaction.

As discussed above, the ability to integrate nanoscale components into an existing circuit remains problematic. Therefore we used the M13 bacteriophage to test whether the self-assembly of the ACID:BASE-C heterodimer can be used for the specific immobilization of a nanoscale component. To this end, we re-created a mutant of M13 displaying the ACID peptide on the pIX coat protein [2] (figure 3). The chimeric ACID-pIX protein had three sequence elements not present in the wild-type equivalent: an N-terminal His-tag for ease of purification of the desired phage particles from wild-type particles also created by the expression construct, the ACID sequence and the flexible linker (SGGSG, see above). This engineered virus was produced successfully using the pCGMT-1b phagemid system [22].

NTA-chromatography was used to isolate phage displaying ACID on at least one pIX coat protein (termed M13pIXACID). We then tested its ability to assemble onto a surface functionalized with a layer of BASE-C using SPR. A bare gold SPR surface was derivatized by exposure to BASE-C resulting in the addition of ~500 RU of peptide. This surface was then blocked by exposure to saturating levels of BLK-C. Increasing concentrations of M13pIXACID phage, determined from their absorbance [26], were then injected across the surface (figure 4(A)). No binding was observed between the BASE-C surface and M13pIXACID at concentrations $<0.05 \times 10^{12}$ virions ml^{-1} , but at higher concentrations a stable SPR response was observed up to a concentration of $\sim 5 \times 10^{12}$ virions ml^{-1} . It was not possible to saturate the response even at the highest phage titre. Fitting a Langmuir isotherm to the plot of concentration against SPR response at 300 s (figure 4(B)) yielded a dissociation constant, K_d , of $\sim 30 \mu\text{M}$. The similarity of the two K_d values (for M13pIXACID and free ACID peptide), along with the lack of binding of the phage to layers of JUN-C and BLK-C (figure S2 available at stacks.iop.org/Nano/23/495304/mmedia), strongly suggest that the M13 phage particle is being immobilized via the self-assembly of the ACID:BASE-C heterodimer on the gold surface.

A final experiment was performed to confirm this. 500 RU of BASE-C was immobilized onto each of two bare gold flow-cells using SPR (figure 4(C)). ACID peptide (1 mg ml^{-1}) was injected across the first flow-cell to form the coiled-coil heterodimer, whilst running buffer was injected across the 2nd flow-cell. M13pIXACID phage (0.5×10^{12} virions ml^{-1}) were then injected across both surfaces. Very little if any phage bound to the surface exposed to ACID peptide, presumably because this treatment forms peptide-peptide coiled-coils and there are few free attachment sites. In contrast, the phage binds to the untreated surface. These results confirm that immobilization is due to M13pIXACID:BASE-C interaction.

Finally, we demonstrate the integration of M13 phage particles onto an array of sub-micrometre-spaced gold electrodes fabricated on a SiO_2 surface, directed by the selective self-assembly of the M13pIXACID:BASE-C heterodimer. To permit selective immobilization on individual electrodes within an array, we developed a molecular masking process inspired by our previous work [23, 24] to functionalize each electrode within the array with either a mixture of BASE-C and BLK-C (50:50) or just BLK-C. Colourimetry, using an alkaline phosphatase coupled to streptavidin and a biotin-conjugated ACID peptide, was carried out to confirm the efficacy of the process (figure 5). The entire electrode array was then exposed to a solution of M13pIXACID phage (at a concentration of 1×10^{11} virions ml^{-1}) and incubated overnight. After washing with water the electrodes were examined via

AFM (figure 6). Electrode images were divided into equally-sized squares and the number of phage within each counted. It was found that the phage particles bound preferentially, by a factor of ~ 100 , to the BASE-C/BLK-C covered electrode. This result clearly shows that the use of the ACID:BASE-C heterodimer can specifically immobilize the M13 bacteriophage to a gold electrode via self-assembly.

4. Conclusion

As our ability to control and synthesize materials structured on the nanoscale becomes more advanced, there is increasing need for new technologies that can position and join functional nanoscale elements into complex integrated circuits, and interface them with the outside world. Here we have described a novel approach using proven self-assembling peptide motifs [2] to this challenge to selectively and specifically assemble nanoscale elements onto patterned surfaces. The ease with which one of the coiled-coil partners can be displayed on the M13 bacteriophage suggests that this method could be readily incorporated into existing M13 bacteriophage methodologies, allowing the assembly of a range of functional materials and devices.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- [1]. Huang Y, Chiang C-Y, Lee SK, Gao Y, Hu EL, Yoreo JD, Belcher AM. *Nano Lett.* 2005; 5:1429–34. [PubMed: 16178252]
- [2]. Sweeney RY, Park EY, Iverson BL, Georgiou G. *Biotechnol. Bioeng.* 2006; 95:539–45. [PubMed: 16897782]
- [3]. Naik RR, Brott LL, Clarson SJ, Stone MO. *J. Nanosci. Nanotechnol.* 2002; 2:95–100. [PubMed: 12908327]
- [4]. Lee S-W, Mao C, Flynn CE, Belcher AM. *Science.* 2002; 296:892–5. [PubMed: 11988570]
- [5]. Dang X, Yi H, Ham M-H, Qi J, Yun DS, Ladewski R, Strano MS, Hammond PT, Belcher AM. *Nature Nanotechnol.* 2011; 6:377–84. [PubMed: 21516089]
- [6]. Lee YJ, Yi H, Kim WJ, Kang K, Yun DS, Strano MS, Ceder G, Belcher AM. *Science.* 2009; 324:1051–5. [PubMed: 19342549]
- [7]. Alivisatos A, Johnsson K, Peng X, Wilson T, Loweth C, Bruchez M, Schultz P. *Nature.* 1996; 382:609–11. [PubMed: 8757130]
- [8]. Wolf E, Kim PS, Berger B. *Protein Sci.* 1997; 6:1179–89. [PubMed: 9194178]
- [9]. Mason JM, Arndt KM. *ChemBioChem.* 2004; 5:170–6. [PubMed: 14760737]
- [10]. Crick F. *Acta Crystallogr.* 1953; 6:689–97.
- [11]. Schuermann M, Hunter JB, Hennig G, Müller R. *Nucleic Acids Res.* 1991; 19:739–46. [PubMed: 1901988]
- [12]. Oakley M, Kim P. *Biochemistry.* 1998; 37:12603–10. [PubMed: 9730833]

- [13]. Graddis TJ, Myszkowski DG, Chaiken IM. *Biochemistry*. 1993; 32:12664–71. [PubMed: 8251485]
- [14]. Reinke AW, Grant RA, Keating AE. *J. Am. Chem. Soc.* 2010; 132:6025–31. [PubMed: 20387835]
- [15]. O'Shea EK, Lumb KJ, Kim PS. *Curr. Biol.* 1993; 3:658–67. [PubMed: 15335856]
- [16]. Stevens MM, Allen S, Sakata JK, Davies MC, Roberts CJ, Tendler SJB, Tirrell DA, Williams PM. *Langmuir*. 2004; 20:7747–52. [PubMed: 15323527]
- [17]. Grünberg R, Ferrar TS, van der Sloot AM, Constante M, Serrano L. *Nucleic Acids Res.* 2010; 38:2645–62. [PubMed: 20215443]
- [18]. Park K, Lee JM, Jung Y, Habtemariam T, Salah AW, Fermin CD, Kim M. *Analyst*. 2011; 136:2506–11. [PubMed: 21519608]
- [19]. White SJ, Morton W, Cheah BC, Bronowska A, Davies AG, Stockley PG, Wälti C, Johnson S. *Langmuir*. 2012; 22:13877–82. [PubMed: 22934624]
- [20]. Campbell K, Lumb K. *Biochemistry*. 2002; 41:7169–75. [PubMed: 12033951]
- [21]. Lumb K, Kim P. *Biochemistry*. 1995; 34:8642–8. [PubMed: 7612604]
- [22]. Gao C, Mao S, Lo CH, Wirsching P, Lerner RA, Janda KD. *Proc. Natl Acad. Sci. USA*. 1999; 96:6025–30. [PubMed: 10339535]
- [23]. Wälti C, Wirtz R, Germishuizen WA, Bailey DMD, Pepper M, Middelberg APJ, Davies AG. *Langmuir*. 2003; 19:981–4.
- [24]. Evans D, Johnson S, Laurenson S, Davies AG, Ferrigno PK, Wälti C. *J. Biol.* 2008; 7(3)
- [25]. van Wezenbeek PM, Hulsebos TJ, Schoenmakers JG. *Gene*. 1980; 11:129–48. [PubMed: 6254849]
- [26]. Day, LA.; Wiseman, RL. *A Comparison of DNA Packaging in the Virions of fd, Xf and Pf1*. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 1978.

ACID: AQLEKELQALEKELAQLEWENQALEKELAQ

ACID-C: AQLEKELQALEKELAQLEWENQALEKELAQGGGSC

BASE-C: AQLKKKLQANKKKLAQLKWKLQALKKKLAQGGGSC

JUN-C: IARLEEKVKTLKAQNSELASTANMLREQVAQLKQKVVMNHGGGSC

BLK-C: SLDTLAEQLDPSANNVLS

C = Cysteine (Functional group = $\text{CH}_2\text{-SH}$)

Figure 1.

Sequences of peptides used. The sequences of ACID, BASE-C, JUN-C and BLK-C are shown as one letter code, and were taken from the following references (ACID, BASE: [15] . JUN-C: [2]). Amino acids (GGGSC) in red were introduced to allow chemoselective immobilization (see text). The asparagines highlighted in green were incorporated to favour formation of anti-parallel coiled-coils.

Figure 2.

SPR investigation of on-surface coiled-coil assembly. (A) 50 μ l of ACID (0.005–1.0 mg mL^{-1} in 0.1 M sodium phosphate, pH 7) were injected over immobilized BASE-C at flow rate 10 μ l min^{-1} . The BASE-C layer had been backfilled with the non-helical blocking peptide, BLK-C. (B) SPR responses 300 s after the injection of ACID fitted with a Langmuir isotherm.

Figure 3.

(A) Schematic diagram of the M13 bacteriophage highlighting the position of the pIX coat protein on which ACID is displayed via the flexible GGGs linker. (B) The amino acid sequence of the chimeric pIX.

Figure 4.

(A) SPR sensorgrams showing the immobilization of M13pIXACID via self-assembly of the ACID:BASE-C heterodimer. 50 μl of M13pIXACID at increasing concentrations were washed over immobilized BASE-C at a flow rate of 10 $\mu\text{l min}^{-1}$. (B) The SPR responses 300 s after the injection of various concentrations of M13pIXACID. (C) Confirmation of specificity of phage immobilization. 1 mg ml^{-1} of ACID peptide (red line) or buffer (black line) were injected across separate flow-cells previously coated with ~ 500 RU of BASE-C for 600 s (Injection 1). A recovery period of flow of buffer alone was followed on both flow-cells by an injection of M13pIXACID (0.5×10^{12} virions ml^{-1}), again for 600 s (Injection 2).

Figure 5.

Specific immobilization of the ACID peptide onto a peptide functionalized gold surface. Optical micrograph showing the results of a colourimetric staining experiment, where the electrode array (functionalized with either BLK-C or a 50:50 mixture of BASE-C/BLK-C, termed BASE-C, as labelled in figure), was incubated with biotin-conjugated ACID-C (via a C-terminal cysteine residue), washed thoroughly with water and then stained using alkaline phosphatase. Scale bar 5 μm , + are the cross-hairs used for alignment during the lithographic process.

Figure 6.

Specific immobilization of the M13pIXACID phage onto functionalized gold electrodes facilitated through the ACID:BASE-C coiled-coil interaction. Gold electrodes fabricated on a SiO₂-capped Si wafer were functionalized with either BLK-C or a 50:50 mixture of BASE-C/BLK-C (as indicated in figure). AFM image showing a functionalized electrode array which was exposed to and incubated with M13pIXACID phage (1×10^{11} virions ml⁻¹ in pH 9 0.1 M sodium phosphate) overnight before rinsing with water. Scale bar equals 1 μ m.