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An Intramolecular O−N Migration Reaction on Gold Surfaces: Toward the Preparation of Well-Defined Amyloid Surfaces

Stephanie Boussert,[†,‡] Ismael Diez-Perez,[§] Marcelo J. Kogan,[⊥] Eliandre de Oliveira,[†,*] and Ernest Giralt[‡,||,*]

[†]Proteomics Platform, Barcelona Science Park, Baldiri Reixac 10, E-08028 Barcelona, Spain, [‡]Institute for Research in Biomedicine, Barcelona Science Park, Baldiri Reixac 10, E-08028 Barcelona, Spain, [§]Department of Chemical Physics, University of Barcelona, Martí i Franques 1, 08028 Barcelona, Spain, [⊥]Department of Pharmacology and Toxicology and Center for Advanced Interdisciplinary Research in Materials (CIMAT), University of Chile, Olivos 1007 Independencia, Santiago de Chile, and [||]Department of Organic Chemistry, University of Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain

ABSTRACT Amyloids are a family of self-aggregating proteins implicated in various central nervous system disorders, including Alzheimer's disease (AD). It is thought that prefibrillar soluble forms of amyloid peptides, including oligomers, may be the main pathogenic factor in AD. Herein we describe the fabrication of well-defined, functionalized, monomeric β-amyloid peptide surfaces for studying protein−protein interactions. We first prepared a nonaggregating analogue of the β-amyloid peptide and then attached it to a gold surface covered with a self-assembled monolayer (SAM) of alkanethiols. After attachment, the native form of the β-amyloid peptide (Aβ40) was obtained by surface-level intramolecular O−N migration. The surface was characterized by atomic force microscopy (AFM) and self-assembled monolayer for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (SAMDI-TOF MS). The interaction between the surface-bound Aβ40 and monoclonal anti-Aβ40 antibody was tracked by AFM and chemiluminescence, which revealed that the Aβ40 was attached mainly in its monomeric form and that the protein−protein complex was assembled on the surface. Last, we used a proteomics approach to demonstrate the specificity of the Aβ40-functionalized surface in surface-binding experiments employing serum amyloid P (SAP) and bovine serum albumin (BSA).

KEYWORDS: β-amyloid · gold surface · protein−protein interaction · self-assembled monolayers for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (SAMDI-TOF-MS) · atomic force microscopy (AFM) · proteomics

Amyloids are a family of self-aggregating proteins related to various central nervous system disorders, including Alzheimer's disease (AD). The two most distinctive brain pathologies of AD are neuritic plaques, which mainly contain insoluble aggregates of the β-amyloid peptide (Aβ), and neurofibrillary tangles of abnormally phosphorylated Tau protein.[1] Cleavage of the β-amyloid precursor protein (APP) by proteases such as β-secretase and γ-secretase produces a predominant product of 40 amino acid residues: β-amyloid peptide (Aβ40).[2] Other Aβ species of different lengths are also produced, including a fibrillogenic peptide of 42 amino acids (Aβ42). The deposition of Aβ40 and Aβ42 into cerebral plaques begins with nucleation of soluble Aβ42 into fibrils, followed by accumulation of soluble Aβ40.[3] In fact, soluble prefibrillar forms of amyloid peptides, including monomers, are suspected to be the main pathogenic factor in AD.[4−7] Albeit the mechanism of Aβ toxicity remains unclear, we believe that learning about how Aβ peptides interact with proteins should enable a better understanding of AD pathogenesis.

At certain crucial concentrations, Aβ peptides strongly tend to aggregate and form fibrils. These concentrations vary with peptide structure and the medium. Moreover, an ensemble of different aggregation states that fall between nucleates and fibrils, called *protofibrils*, are present in solution. This heterogeneity in the assembly states of Aβ peptides and in their aggregating behavior complicates the study in solution of Aβ structure and of protein−protein interactions involving Aβ. Therefore, the development of a technique to obtain intact and soluble Aβ monomers under physiological conditions is crucial for being able to study their role in AD pathogenesis. Immobilizing molecules on surfaces is a useful technique for studying protein−protein interactions. However, synthesis and immobilization of Aβ are very difficult due to its strong tendency to self-aggregate and form heterogeneous species. Various approaches have been developed for synthetically challenging peptides (*e.g.*, those that are strongly hydrophobic or tend to aggregate). Solid phase peptide synthesis (SPPS) is a known technique for obtaining peptides in small to medium amounts.[8] One of the

*Address correspondence to ernest.giralt@irbbarcelona.org, eoliveira@pcb.ub.cat.

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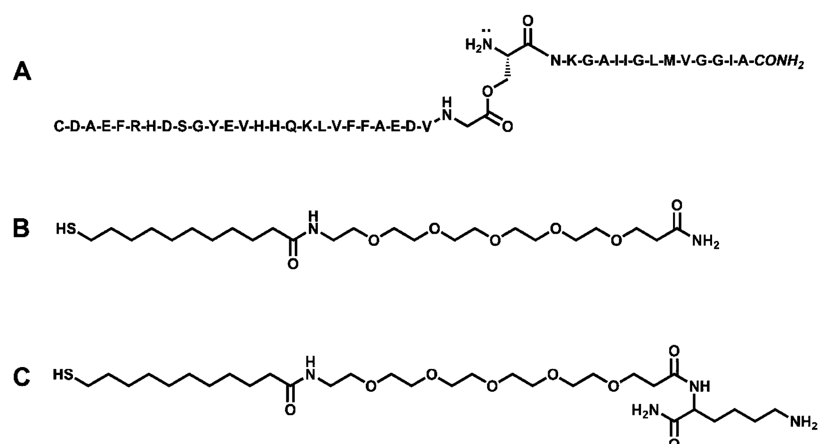

Figure 1. (A) Sequence of the Cys-Aβ40 isopeptide; (B) sequence of the penta-EGylated alkanethiol; (C) sequence of the *N*-Lys-(penta-EGylated) alkanethiol.

methods proposed to solve the problem of aggregation of hydrophobic peptide during synthesis entails the use of special building blocks.[9] More recently, Sohma *et al.* showed that using an *O*-acyl residue instead of an *N*-acyl residue in the peptide sequence could overcome the solubility problems of large peptides with difficult sequences.[10] The resulting *O*-acyl isopeptide can then be easily converted to the native peptide under physiological conditions (pH 7.4) *via* a quick, one-way intramolecular *O*−*N*-acyl migration reaction. Because of the convenient SPPS reaction conditions, we chose this last method to synthesize the soluble Aβ40 in the present work.

Protein−protein interactions are typically studied in solution. Nevertheless, functionalized surfaces such as microarrays and microchips have sparked research on the interactions of proteins and their complexes with other proteins and with peptides.[11−14] Self-assembled monolayers (SAMs) are highly effective for modifying surfaces indicated for these applications[15,16] and enable immobilization of macromolecules, including DNA,[17] proteins,[18,19] and cells.[20,21] SAMs of alkanethiols on gold (111) are probably the most studied class and provide a dense, well-defined, and readily functionalizable monolayer.[22] For instance, alkanethiols can be modified with oligoethylene or polyethylene glycol (EG) groups, which are widely used to generate inert surfaces because they confer high resistance to nonspecific adsorption of biomolecules or cells to surfaces.[23,24] However, the synthesis of EGylated alkanethiols is challenging by conventional organic chemistry.[7]

Surface characterization of SAMs by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (SAMDI-TOF MS) is a powerful new method. It has been employed in enzymatic activity assays,[25] screening of peptide libraries,[26] and characterization of products generated by electrochemical reactions.[27] It is also utile for verifying surface immobilization of a ligand.[28] Other biophysical approaches such as atomic force microscopy (AFM) enable visualization of individual proteins and their complexes on surfaces in near native conditions, which preserves their biological structure.[29−31]

Herein we report a strategy for obtaining and characterizing functionalized surfaces with a monomeric form of Aβ40 and demonstrate the utility of these surfaces for studying protein−protein interactions.

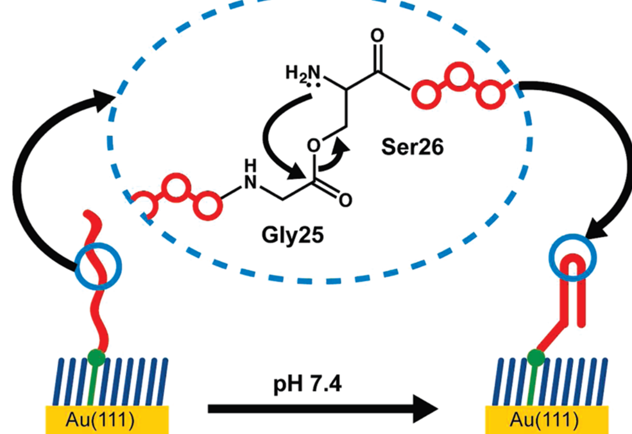

Figure 2. Schematic representation of the intramolecular *O*−*N*-acyl migration of Aβ40 on the functionalized gold surface.

RESULTS AND DISCUSSION

In a first step, we synthesized Aβ40 isopeptide, a nonaggregating precursor of Aβ40, as well as the two penta-EGylated alkanethiols, *via* solid phase synthesis (Figure 1). To assemble the SAM, we covered the gold surface with a mixture (2:1) of penta-EGylated alkanethiol and *N*-Lys-(penta-EGylated) alkanethiol dissolved in dimethylformamide (DMF). We then immobilized the amyloid isopeptide onto the SAM and then performed a quick, one-way intramolecular *O*−*N*-acyl migration to obtain the native Aβ40 on surface. We used the complementary techniques of atomic force microscopy (AFM) and mass spectrometry (MS) to characterize the functionalized gold surface and to track the interaction between the surface-bound Aβ40 and a model protein.

Synthesis of Aβ40 Isopeptide. The peptide was synthesized on solid phase using an Fmoc strategy and the *O*-acyl isopeptide methodology.[32] This method is advantageous in that it affords soluble Aβ40, which enables chromatographic purification and further studies in solution. For specific attachment of Aβ40 to our SAM, we added an N-terminal cysteine to the peptide sequence (Figure 1A). Since the N-terminus of the amyloid peptide sequence is structurally disordered, we reasoned that surface attachment *via* this terminus would not disrupt peptide folding.

Functionalization of the Aβ40 Surface. The functionalized surface was constructed in three steps. In the first step,

a gold surface was covered with SAMs of two penta-EGylated alkanethiols, one of which contained a binding site for the cysteine-containing Aβ40 isopeptide (Cys-Aβ40 isopeptide) (Figure 1B). Synthesis of these alkanethiols was greatly simplified on solid phase: only two or three synthetic steps were required (depending on the incorporation of the peptide binding site). The desired SAM was formed by incubation of a mixed solution of these two modified alkanethiols on the gold surface. In the second step, the free amino group of the lysine side chain was reacted with a bifunctional 3-(maleimido)propionic acid *N*-hydrosuccinimide ester linker.[28] Finally, the Cys-Aβ40 isopeptide was immobilized onto the SAM in acid solution to prevent intramolecular *O*−*N*-acyl migration and to enable surface binding of the Aβ40 isopeptide (primarily in monomeric form). Following immobilization of the Aβ40 isopeptide, the surface was washed and incubated with PBS to remove traces of acid solution, leave the surface in physiological conditions, and initiate the on-surface intramolecular *O*−*N*-acyl migration (Figure 2).

Characterization of the Aβ40 Surface. This Aβ40-functionalized gold surface was then analyzed by AFM (Figure 3A1). AFM analysis showed a background of a compact and dense monolayer corresponding to the adsorption of the mixed SAM. We also observed many bright spots on the surface corresponding to the attached Aβ40 (Figure 3A2). The measured height of each spot was *ca.* 6 nm (Figure 3A3). As the theoretical length of the Cys-Aβ40 in a single β-strand is 13.4 nm, this result suggested that our Cys-Aβ40 adopts a β-hairpin conformation and that this surface-bound version of the *O*−*N* intramolecular acyl migration reaction was successful. The experimental height is in good accordance with the dimensions given in the structural model for Aβ40 molecules (6 nm).[33] The homogeneity in the measured heights of the bright spots confirmed our hypothesis that the Aβ40 is mainly attached to the surface in monomeric form. Moreover, the homogeneous distribution of the Aβ40 on the surface with no large-scale domain separation indicates that molecular segregation of the two modified alkanethiols does not occur during formation of the mixed SAM. The same functionalized gold surface was also characterized by MS. We were able to directly confirm the presence of the Aβ40 on the surface using SAMDI-TOF-MS (Figure 4).

Protein−Protein Interaction Studies. We then performed a protein−protein interaction assay directly on the surface, which we tracked by AFM and chemiluminescence. AFM is advantageous in its ability to capture images of biological samples in solution and thus preserve the native structure of protein complexes.

Our Aβ40-functionalized gold surface was incubated with an anti-Aβ monoclonal antibody (anti-Aβ) described to react with amino acid residues 1−17 of human Aβ. AFM measurements showed a clear change in the surface morphology after antibody incubation (Figure 3B2). Topographic profiles revealed heights from 17 to 23 nm (Figure 3B3), which indicate the formation of an Aβ40/anti-Aβ complex. These measurements are in close agreement with the dimensions of IgG antibodies.[34,35] All of the AFM measurements were made in solution, which allow free rotation of the surface-bound Aβ. Therefore, the range of heights observed suggests that the highest and lowest spots on the AFM images correspond to antigen−antibody complexes in standing pose and in lying pose, respectively

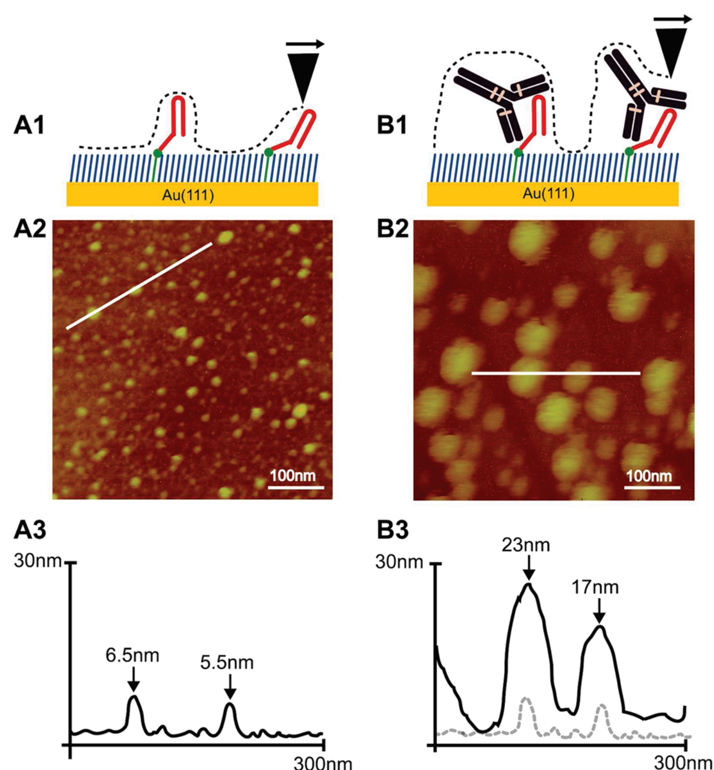

Figure 3. Schematic representation of AFM study of the Aβ40-functionalized gold surface before (A1) and after (B1) incubation with the monoclonal anti-Aβ antibody; AFM images of the Aβ40-functionalized gold surface before (A2) and after (B2) incubation; surface morphology measurements (AFM) of the Aβ40-functionalized gold surface before (A3) and after (B3) incubation.

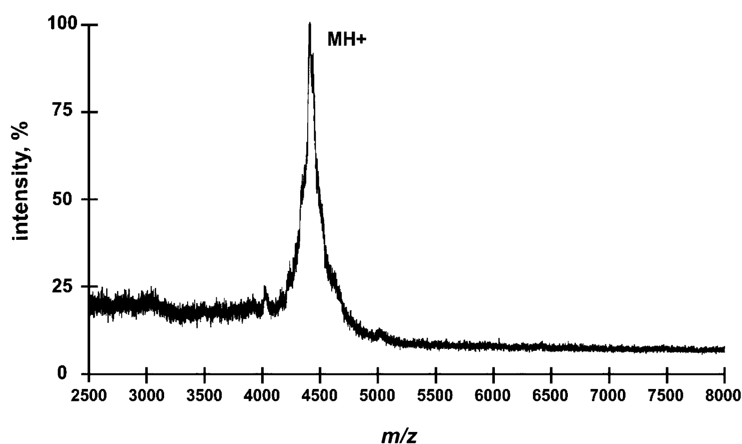

Figure 4. SAMDI-TOF-MS spectrum of the Aβ40-functionalized gold surface.

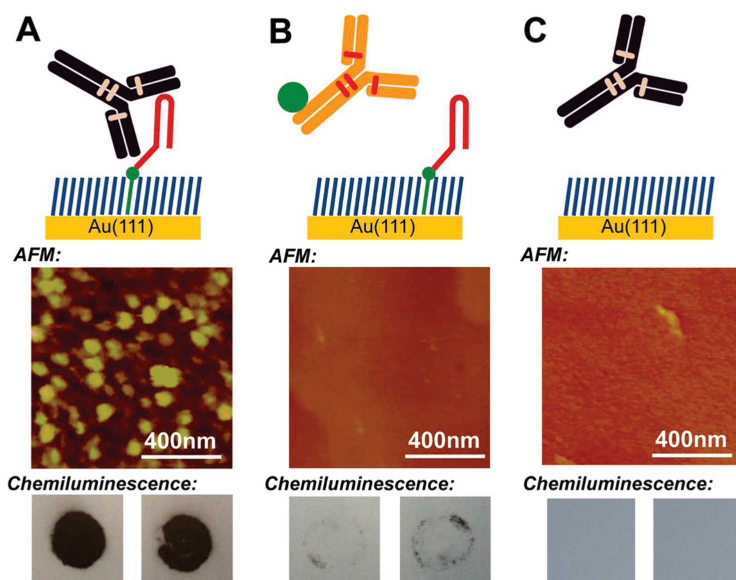

Figure 5. AFM and chemiluminescence experiments. (A) AFM images of an Aβ40-functionalized gold surface after incubation with the monoclonal anti-Aβ antibody; resulting light was detected by chemiluminescence after incubation with a secondary antibody labeled with peroxidase, indicating the presence of the monoclonal anti-Aβ antibody on the surface; (B) AFM images of an Aβ40-functionalized gold surface after incubation with the secondary antibody (labeled with peroxidase); no resulting light was detected after chemiluminescence reaction; (C) AFM images of a penta-EGylated alkanethiol-functionalized gold surface after incubation with the monoclonal anti-Aβ antibody; no resulting light was detected after incubation with a secondary antibody labeled with peroxidase and subsequent chemiluminescence reaction.

(Figure 3B1). This Aβ40/anti-Aβ interaction was also directly observed on the surface by chemiluminescence. After incubation with a secondary antibody labeled with peroxidase, chemiluminescence was detected on autoradiography film, indicating the presence of anti-Aβ (Figure 5A). Ensuring that the observed interactions were specific and avoiding nonspecific interactions with the modified surface was critical to our study. To confirm the specificity of the antigen−antibody interaction on surface, we then incubated an identical Aβ40 surface with a secondary antibody labeled with peroxidase enzyme. The AFM images resulting from the analysis of the surface did not show any evidence of the formation of a nonspecific, Aβ-secondary antibody complex (Figure 5B). Moreover, no resulting light was detected by chemiluminescence. In contrast, and as expected, when we incubated a monolayer surface containing only the penta-EGylated alkanethiols (1-11-mercaptoundecanamido-3,6,9,12,15-pentaoxaoctadecan-18-amide) with anti-Aβ, we did not detect any nonspecific interactions either by AFM or by chemiluminescence (Figure 5C). These observations validated the ability of our penta-EGylated alkanethiols to prevent nonspecific adsorption of proteins onto the surface.

We then evaluated the ability of our Aβ40 surface to identify protein interactions by using a proteomics approach. First, we used serum amyloid P (SAP) component, a 23 kDa protein known to interact with Aβ.[36,37] SAP has been located in neurofibrillar tangles, senile plaques, and amyloid angiopathy of the Alzheimer's disease. SAP aggregates have also been observed in other neurodegenerative diseases, including Creutzfeldt−Jakob disease, Pick's disease, Parkinson's disease, and Lewy body disease.[38−40] SAP is also known to promote plaque formation by coaggregating with Aβ in the presence of Ca^{2+}.[41] Furthermore, SAP is commercially available, making it a convenient model for evaluating the ability of our Aβ40-functionalized gold surface to capture proteins that interact with Aβ and allow on-chip enzymatic digestion for MS analysis. We performed a MS study of protein−protein interactions whereby only the target protein was incubated on the surface (Figure 6A). The signals observed in the MALDI-MS spectra correspond to the peptide mass fingerprint (PMF) of the SAP protein (Figure 6B). These

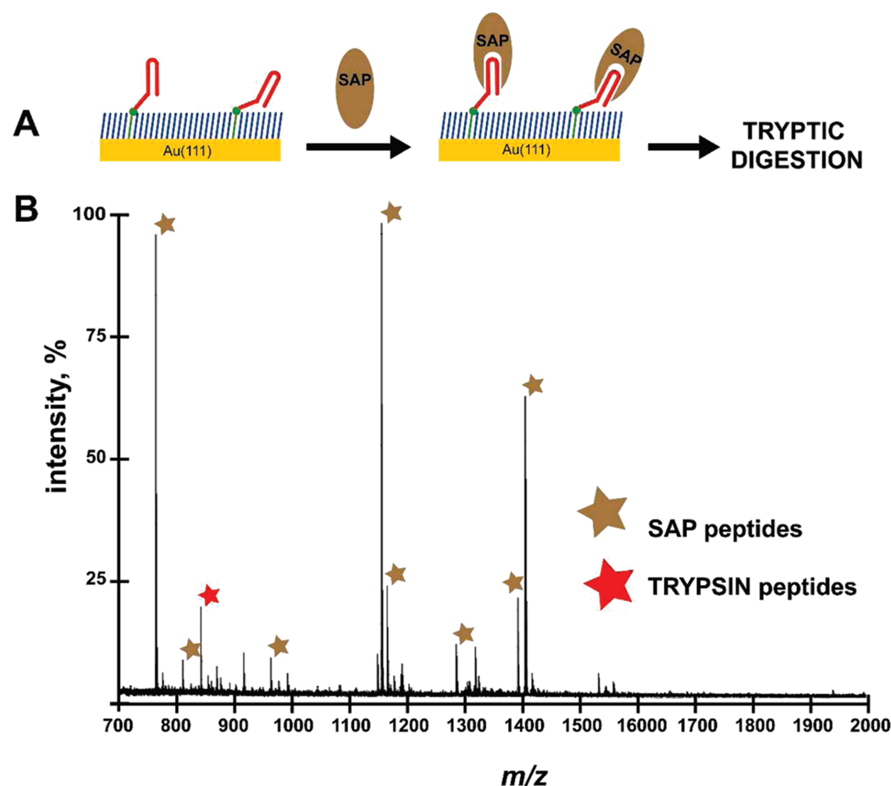

Figure 6. (A) Serum amyloid P (SAP) was incubated on the Aβ40-functionalized gold surface and then digested on the surface with trypsin; (B) MALDI-MS spectrum of tryptic digested peptides after incubation of SAP onto the surface. The brown stars indicate SAP signals, and the red star indicates a peptide from trypsin autolysis.

TABLE 1. Data from the Identification of Serum Amyloid P (SAP) by LC-MS/MS

protein	accession number	Mascot score[a]	number of unique peptides	peptide sequence
serum amyloid P component	gi|4502133	281	6	AYSDLSR
				AYSLFSYNTQGR
				DNELLVYK
				VGEYSLYIGR
				QGYFVEAQPK
				IVLGQEQDSYGGK

[a]MASCOT score >43 indicates high homology to *p* value < 0.05 (probability 95%).

data indicate the presence of SAP on the surface and confirm its interaction with Aβ40. To check the specificity of the SAP-Aβ40 interaction, we performed the same experiment in the presence of bovine serum albumin (BSA), a 66 kDA protein widely used as a control in proteomics experiments. After incubation, washing, and trypsin digestion, the peptides were analyzed by online liquid chromatography tandem mass spectrometry (LC-MS/MS). The LC-MS/MS analysis revealed only tryptic SAP peptides (Table 1), thereby demonstrating the specificity of the SAP−Aβ40 interaction. Despite the fact that BSA is easily identified by MS—because it generates numerous peptide species upon proteolytic digestion—no peptides corresponding to the trypsin digestion of BSA were observed.

In summary, the methodology reported here represents an important step toward the preparation of well-defined, functionalized surfaces of monomeric Aβ and opens the way to general preparation of surfaces modified with other proteins that are prone to aggregation. Having the support of both AFM and MS data adds to the robustness of the methodology. We have shown that our Aβ surface is functionally active and is compatible with MS techniques for protein−protein interactions studies. As indicated by the presence of polyethylene glycol in the SAM, only specific interactions are observed. Moreover, the system provides a convenient and sensitive range that allows classical proteomics assays such as enzymatic digestions and identification *via* MS. We believe that the utility of the surfaces prepared here will be invaluable to the future study of specific protein−protein interactions by proteomic MS studies.

METHODS

Preparation of the Gold Surface: The crystalline gold (111) surface was cleaned and flattened using electrochemistry (Au cathode and a Pt anode; 45 s in 0.1 M H_2SO_4; voltage fixed at 10 V) whereby thick, hydrous gold(III) oxide was formed. The oxide was washed away using MQ H_2O followed by immersion in 1 M HCl solution (1 min) to afford a fresh metallic gold surface. The surface was then washed again with MQ water before being annealed in a butane/propane flame (3 min) to increase the terrace size and remove any volatile contaminants. The surface was cooled under Ar for at least 15 min and then immediately used. After being annealed, the surface exhibited atomically smooth gold (111) terraces of about 1 μm wide, separated by monatomic steps in height.[42]

Functionalization of the Gold Surface: A freshly prepared solution of a mixture of the penta-EGylated alkanethiol and the *N*-Lys-(penta-EGylated) alkanethiol (1 μM in DMF, ratio 2:1 v/v for Aβ/antiAβ experiments and 9:1 v/v for Aβ/SAP experiments) was incubated on the freshly prepared gold surface for 14 h. The surface was then thoroughly rinsed with DMF to remove any unbound modified alkanethiols, dried under a strong stream of N_2, and then immediately incubated with a freshly prepared solution of maleimide bifunctional group: 3-(maleimido)propionic acid *N*-hydrosuccinimide ester (10 μM in DMF, room temperature, 3.5 h, with agitation 200 rpm). The surface was then rinsed thoroughly with DMF and dried under a strong stream of N_2. An acidic aqueous solution of Cys-βA40 (0.5 μM, 0.1% TFA) was added to the surface and incubated for 2 h at 4 °C. The surface was then washed with 0.1% aqueous TFA. Prior to AFM analysis, it was incubated for at least 30 min with PBS (pH 7.4). After AFM imaging, the surface was rinsed with PBS and then incubated with a monoclonal anti-human β-amyloid antibody (1 μL/60 μL PBS−0.1% Tween20, overnight, 4 °C). After incubation, the surface was thoroughly rinsed with PBS−0.1% Tween20 (*ca.* 20 mL) and immediately analyzed by AFM.

AFM Experiments: Imaging was performed with a MultiMode instrument controlled by Nanoscope IV electronics (Digital Instruments, Santa Barbara, CA) equipped with either a 12 m scanner (E-scanner) or a 120 m scanner (J-scanner). All images were recorded in solution (PBS) in tapping mode using a liquid cell without the O-ring seal.

SAMDI-MS Experiments: A saturated solution of α-cyano-4-hydroxycinnamic acid (5 mg/mL in 0.1% TFA/MeCN 1:1, v/v) was deposited directly onto the Aβ40-functionalized gold surface, which was then analyzed by mass spectrometry. MS analysis was performed on a Voyager DE-RP (Applied Biosystem) in positive linear mode with accelerating time of 25 000 V, grid voltage of 92%, and extraction delay time of 300 ns.

Chemiluminescence Experiments: After AFM analysis, the functionalized gold surface was rinsed with PBS and incubated with anti-mouse IgG1 secondary antibody horseradish peroxidase (1 μL in a 5 mL mixture of PBS 98.9%, Tween20 0.1%, and nonfat milk 1% at room temperature for 1 h). The gold surface was washed with PBS−Tween20 and incubated for 1 min with detection reagents following manufacturer instructions. This elicits peroxidase-catalyzed oxidation of luminol and subsequently, enhanced chemiluminescence, whereby the peroxidase-labeled antibody is bound to the antigen on the primary antibody. The resulting light was detected on autoradiography films at 1 and 3 min.

On-Gold Incubation and Digestion: The β-amyloid gold surface was incubated for 2 h at 4 °C with a solution of SAP protein (0.5 μg/μL, in buffer 140 mM NaCl, 10 mM TrisHCl, 0.1% NaN_3, 2 mM $CaCl_2$ buffer) or with a mixed solution of SAP and BSA proteins (0.4 μg/μL for each protein, ratio 50:50 v/v, in buffer 140 mM NaCl, 10 mM TrisHCl, 0.1% NaN_3, 2 mM $CaCl_2$ buffer). After the washing step (3 × 5 mL of binding buffer and 3 × 5 mL water), the surface was subjected to a tryptic digestion during 5 h at 37 °C with 50−100 ng of trypsin in 50 mM ammonium bicarbonate. The released peptides were extracted in 50% acetonitrile−0.1% TFA and dried.

MALDI-MS Analysis: The peptides were redissolved in 0.1% TFA, passed through a RP-C18 microcolumn to remove salts, and eluted with matrix solution (3 μL of a solution of 5 mg α-cyano-4-hydroxycinnamic acid in 1 mL of 50% acetonitrile−0.1% TFA) onto a MALDI plate. MS spectra were acquired in positive reflector mode (voltage of 20 kV in the source 1 and laser intensity ranged from 5800 to 6200). Typically, 500 shots per spectrum were accumulated. MALDI-MS analysis was performed on a

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MALDI-TOF/TOF (4700 Proteomics Analyzer, Applied Biosystems).

LC-MS/MS Analysis: The tryptic digested peptide samples were redissolved in 15 μL of 1% formic acid solution, and 4 μL was injected for chromatographic separation using a reverse-phase capillary C_{18} column (PepMap column, LC Packings; internal diameter = 75 μm; length = 15 cm). The eluted peptides were ionized *via* coated nano-ES needles (PicoTip, New Objective). A capillary voltage of 2000 V was applied together with a cone voltage of 80 V. The collision in the CID (collision-induced dissociation) was 20−35 eV, and argon was employed as collision gas. LC-MS/MS analysis was performed on a Cap-LC-nano-ESI-Q-TOF (CapLC, Micromass-Waters). Data were generated in PKL file format.

Database Search: The PKL file was run through the MASCOT search engine against the nonredundant NCBI database. The search parameters were 1 missed cleavage, fixed, and variable modifications were carbamidomethyl of cystein and oxidation of methionine, respectively. Peptide tolerance was 100 ppm and 0.25 Da, respectively, for MS and MS/MS spectra.

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Supporting Information Available: Materials, synthesis of 11-(tritylthio)undecanoic acid, synthesis of the penta-EGylated alkanethiol 1-(11-mercaptoundecanamido)-3,6,9,12,15-pentaoxaoctadecan-18-amide, synthesis of the *N*-Lys-(penta-EGylated) alkanethiol (*N*-(1,6-diamino-1-oxohexan-2-yl)-1-(11-mercaptoundecanamido)-3,6,9,12,15-pentaoxaoctadecan-18-amide), synthesis of Cys-Aβ40 click peptide, preparation of the gold surface, functionalization of the gold surface, AFM experiments, MS experiments. This material is available free of charge *via* the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

1. Selkoe, D. J. Alzheimer's Disease: Genes, Proteins, and Therapy. *Physiol. Rev.* **2001**, *81*, 741–766.
2. Wolfe, M. S. Therapeutic Strategies for Alzheimer's Disease. *Nat. Rev. Drug Discovery* **2002**, *1*, 859–866.
3. Rottkamp, C. A.; Atwood, C. S.; Joseph, J. A.; Nunomura, A.; Perry, G.; Smith, M. A. The State *versus* Amyloid-Beta: The Trial of the Most Wanted Criminal in Alzheimer's Disease. *Peptides* **2002**, *23*, 1333–1341.
4. Ferreira, S. T.; Vieira, M. N.; De Felice, F. G. Soluble Protein Oligomers as Emerging Toxins in Alzheimer's and Other Amyloid Diseases. *IUBMB Life* **2007**, *59*, 332–345.
5. Glabe, C. C. Amyloid Accumulation and Pathogenesis of Alzheimer's Disease: Significance of Monomeric, Oligomeric and Fibrillar A-Beta. *Subcell. Biochem.* **2005**, *38*, 167–177.
6. Haass, C.; Selkoe, D. J. Soluble Protein Oligomers in Neurodegeneration: Lessons from the Alzheimer's Amyloid Beta-Peptide. *Nat. Rev. Mol. Cell. Biol.* **2007**, *8*, 101–112.
7. Walsh, D. M.; Selkoe, D. J. A-Beta Oligomers—A Decade of Discovery. *J. Neurochem.* **2007**, *101*, 1172–1184.
8. Merrifield, R. B. J. Solid Phase Peptide Synthesis. The Synthesis of a Tetrapeptide. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154.
9. Mutter, M.; Nefzi, A.; Sato, T.; Sun, X.; Wahl, F.; Wohr, T. Pseudo-Prolines (psi pro) for Accessing "Inaccessible" Peptides. *Pept. Res.* **1995**, *8*, 145–153.
10. Sohma, Y.; Hayashi, Y.; Skwarczynski, M.; Hamada, Y.; Sasaki, M.; Kimura, T.; Kiso, Y. O−N Intramolecular Acyl Migration Reaction in the Development of Prodrugs and the Synthesis of Difficult Sequence-Containing Bioactive Peptides. *Biopolymers* **2004**, *76*, 344–356.
11. Kodadek, T. Protein Microarrays: Prospects and Problems. *Chem. Biol.* **2001**, *8*, 105–115.
12. Cahill, D. J. Protein and Antibody Arrays and their Medical Applications. *J. Immunol. Methods* **2001**, *250*, 81–91.
13. Wilson, D. S.; Nock, S. Functional Protein Microarray. *Curr. Opin. Chem. Biol.* **2002**, *6*, 81–85.
14. Zhu, H.; Snyder, M. Protein Chip Technology. *Curr. Opin. Chem. Biol.* **2003**, *7*, 55–63.
15. Mrksich, M.; Whitesides, G. M. Using Self-Assembled Monolayers to Understand the Interactions of Man-Made Surfaces with Proteins and Cells. *Annu. Rev. Biophys. Biomol. Struct.* **1996**, *25*, 55–78.
16. Love, J. C.; Estroff, L. A.; Kriebel, J. K.; Nuzzo, R. G.; Whitesides, G. M. Self-Assembled Monolayers of Thiolates on Metals as a Form of Nanotechnology. *Chem. Rev.* **2005**, *105*, 1103–1169.
17. Frutos, A. G.; Liu, Q.; Thiel, A. J.; Sanner, A. M.; Condon, A. E.; Smith, L. M.; Corn, R. M. Demonstration of a Word Design Strategy for DNA Computing on Surfaces. *Nucleic Acids Res.* **1997**, *25*, 4748–4757.
18. Sigal, G. B.; Bamdad, C.; Barberis, A.; Strominger, J.; Whitesides, G. M. A Self-Assembled Monolayer for the Binding and Study of Histidine-Tagged Proteins by Surface Plasmon Resonance. *Anal. Chem.* **1996**, *68*, 490–497.
19. Muller, W.; Ringsdorf, H.; Rump, E.; Wildburg, G.; Zhang, X.; Angermaier, L.; Knoll, W.; Liley, M.; Spinke, J. Attempts to Mimic Docking Processes of the Immune System: Recognition Induced Formation of Protein Multilayers. *Science* **1993**, *262*, 1706–1708.
20. Mrksich, M.; Chen, C. S.; Xia, Y.; Dike, L. E.; Ingber, D. E.; Whitesides, G. M. Controlling Cell Attachment on Contoured Surfaces with Self-Assembled Monolayers of Alkanethiolates on Gold. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10775–10778.
21. Houseman, B. T.; Mrksich, M. The Microenvironment of Immobilized Arg-Gly-Asp Peptides is an Important Determinant of Cell Adhesion. *Biomaterials* **2001**, *22*, 943–955.
22. Vericat, C.; Vela, M. E.; Salvarezza, R. C. Self-Assembled Monolayers of Alkanethiols on Au(111): Surface Structures, Defects and Dynamics. *Phys. Chem. Chem. Phys.* **2005**, *7*, 3258–3268.
23. Prime, K. L.; Whitesides, G. M. Self-Assembled Organic Monolayers: Model Systems for Studying Adsorption of Proteins at Surfaces. *Science* **1991**, *252*, 1164–1167.
24. Siegel, R. R.; Harder, P.; Dahint, R.; Grunze, M.; Josse, F.; Mrksich, M.; Whitesides, G. M. On-Line Detection of Nonspecific Protein Adsorption at Artificial Surfaces. *Anal. Chem.* **1997**, *69*, 3321–3328.
25. Min, D. H.; Su, J.; Mrksich, M. Profiling Kinase Activities by Using a Peptide Chip and Mass Spectrometry. *Angew. Chem., Int. Ed.* **2004**, *43*, 5973–5977.
26. Min, D. H.; Tang, W. J.; Mrksich, M. Chemical Screening by Mass Spectrometry to Identify Inhibitors of Anthrax Lethal Factor. *Nat. Biotechnol.* **2004**, *22*, 717–723.
27. Yeo, W. S.; Mrksich, M. Electroactive Substrates that Reveal Aldehyde Groups for Bio-Immobilization. *Adv. Mater.* **2004**, *16*, 1352–1356.
28. Houseman, B. T.; Gawalt, E. S.; Mrksich, M. Maleimide-Functionalized Self-Assembled Monolayers for the Preparation of Peptide and Carbohydrate Biochips. *Langmuir* **2003**, *19*, 1522–1531.
29. Yang, J.; Mou, J.; Shao, Z. Molecular Resolution Atomic Force Microscopy of Soluble Proteins in Solution. *Biochim. Biophys. Acta* **1994**, *1199*, 105–114.
30. Scheuring, S. AFM Studies of the Supramolecular Assembly of Bacterial Photosynthetic Core-Complexes. *Curr. Opin. Chem. Biol.* **2006**, *10*, 387–393.
31. Scheuring, S.; Boudier, T.; Sturgis, J. N. From High-Resolution AFM Topographs to Atomic Models of Supramolecular Assemblies. *J. Struct. Biol.* **2007**, *159*, 268–276.
32. Sohma, Y.; Hayashi, Y.; Kimura, M.; Chiyomori, Y.; Taniguchi, A.; Sasaki, M.; Kimura, T.; Kiso, Y. The 'O-Acyl Click-Peptide Method' for the Synthesis of Difficult Sequence-Containing Peptides: Application to the Synthesis of Alzheimer's Disease-Related Amyloid Beta Peptide (Abeta) 1−42. *J. Pept. Sci.* **2005**, *11*, 441–451.

33. Petkova, A. T.; Ishii, Y.; Balbach, J. J.; Antzutkin, O. N.; Leapman, R. D.; Delaglio, F.; Tycko, R. A. Structural Model for Alzheimer's Beta-Amyloid Fibrils Based on Experimental Constraints from Solid State NMR. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16742–16747.
34. Yang, J.; Mayer, M.; Kriebel, J. K.; Garstecki, P.; Whitesides, G. M. Self-Assembled Aggregates of IgGs as Templates for the Growth of Clusters of Gold Nanoparticles. *Angew. Chem., Int. Ed.* **2004**, *43*, 1555–1558.
35. Thomson, N. H. The Substructure of Immunoglobulin G Resolved to 25 kDa Using Amplitude Modulation AFM in Air. *Ultramicroscopy* **2005**, *105*, 103–110.
36. Liko, I.; Mak, M.; Klement, E.; Hunyadi-Gulyas, E.; Pazmany, T.; Medzihradszky, K. F.; Urbanyi, Z. Evidence for an Extended Interacting Surface Between Beta-Amyloid and Serum Amyloid P Component. *Neurosci. Lett.* **2007**, *412*, 51–55.
37. Urbanyi, Z.; Laszlo, L.; Tomasi, T. B.; Toth, E.; Mekes, E.; Sass, M.; Pazmany, T. Serum Amyloid P Component Induces Neuronal Apoptosis and Beta-Amyloid Immunoreactivity. *Brain Res.* **2003**, *988*, 69–77.
38. Kalaria, R. N.; Grahovac, I. Serum Amyloid P Immunoreactivity in Hippocampal Tangles, Plaques and Vessels: Implications for Leakage Across the Blood−Brain Barrier in Alzheimer's Disease. *Brain Res.* **1990**, *516*, 349–353.
39. Kalaria, R.; Galloway, P. G. Widespread Serum Amyloid P Immunoreactivity in Cortical Amyloid Deposits and the Neurofibrillary Pathology of Alzheimer's Disease and Other Degenerative Disorders. *Neuropathol. Appl. Neurobiol.* **1991**, *17*, 189–201.
40. Perlmutter, L. S.; Barrón, E.; Myers, M.; Saperia, D.; Chui, H. C. Localization of Amyloid P Component in Human Brain: Vascular Staining Patterns and Association with Alzheimer's Disease Lesions. *J. Comp. Neurol.* **1995**, *352*, 92–105.
41. Hamazaki, H. Ca(2+)-Dependent Binding of Human Serum Amyloid P Component to Alzheimer's Beta-Amyloid Peptide. *J. Biol. Chem.* **1995**, *270*, 10392–10394.
42. Oncins, G.; Vericat, C.; Sanz, F. Mechanical Properties of Alkanethiol Monolayers Studied by Force Spectroscopy. *J. Chem. Phys.* **2008**, *128*, 044701–044709.