

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 13-250606- -5	FECHA: 2015-03-10 17:20:43
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 7
ACT: 430 REQUERSOLICITA	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
SILVANA MARÍA LÓPEZ DÍAZ

Asunto: Radicación: 13-250606- -5
 Trámite: 11
 Evento: 378
 Actuación: 430
 Oficio: 2600
 Folios: 7

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/US2012/030223				
Fecha de Presentación Internacional	23/03/2012				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado No. 13-250606-00000-0000	22/Octubre/2013
Reivindicaciones:	Radicado No. 13-250606-00000-0000	22/Octubre/2013
Dibujos:	Radicado No. 13-250606-00000-0000	22/Octubre/2013
Prioridad:	US 61/467,256	24/Marzo/2011
	US 61/491,717	31/Mayo/2011
	US 61/583,881	06/Enero/2012

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del Capítulo Primero del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. . Para la presente solicitud se estudia el capítulo reivindicatorio completo (Rad. 13-250606-00000-0000).

Título de la solicitud: “Métodos de diagnóstico y de tratamiento usando una genoteca de Ligandos”.

El problema planteado en esta solicitud consiste en la necesidad de encontrar biomarcadores asociados a enfermedad y a una respuesta de un fármaco.

Para resolver este problema técnico la solicitud en estudio proporciona un método para diagnosticar una respuesta inducida por fármaco en un sujeto.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07K 17/14.

4. No es una invención (Art 15 D486 literal (c))

El objeto de la reivindicación 97 no se considera una invención puesto que hace referencia un marcador identificado mediante el método reclamado. Los marcadores identificados mediante el método reclamado son biomarcadores o moléculas biológicas de una muestra de un sujeto, por lo tanto, se trata de material biológico aislado.

5. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 43 – 45, 50 y 51 no es patentable de acuerdo con el Art citado puesto que hacen referencia a métodos de tratamiento en humanos.

6. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

- 1) Las reivindicaciones de procedimiento deben referirse a unas actividades organizadas en una serie de pasos de manera determinada. El método de diagnóstico de una respuesta inducida por fármaco en un sujeto de la reivindicación 1 no es claro porque está definido en términos de un resultado a alcanzar. Se menciona un paso de selección de una genoteca contra una muestra pero no se definen la manera en que se llevará a cabo dicha selección y que cuales son los criterios para su selección. El paso de la identificación de las características de unión de los marcadores de la muestra con los ligandos de la genoteca tampoco revela las técnicas necesarias para dicha identificación. Tampoco hay certeza de la forma en que se puede determinar una asociación entre los marcadores y la respuesta inducida por fármacos. Otra cosa es que si bien es claro que se trabaja con la muestra de un sujeto, de forma como está redactada la reivindicación no es posible tener clara las condiciones necesarias para que el método se pueda llevar a cabalidad ni se indican los instrumentos necesarios. Se sugiere hacer las correcciones necesarias teniendo en cuenta la materia divulgada en la descripción. Se sugieren también tener en cuenta estas recomendaciones para las reivindicaciones 46 y 52. Además no existe una clara indicación de que se trate de un método in vitro.
- 2) En el capítulo reivindicatorio no se debe hacer mención a figuras, tablas o cuadros de la descripción ya que debe ser completamente claro por sí mismo (reivindicación 3). Se sugiere hacer las correcciones necesarias.
- 3) El paso de determinar si dicho sujeto responde o no a un tratamiento con un fármaco (reivindicación 4) también está definido como un resultado a alcanzar, pues dicha determinación es lo que se quiere lograr pero no se indica la forma en que se va a determinar.
- 4) Las reivindicaciones 5 – 10 y 73 – 77 no son características propias de los métodos reclamados y constituyen una aplicación del mismo. Se recuerda a la solicitante que las reivindicaciones dependientes definen las características adicionales del objeto reclamado en

la reivindicación independiente y que las reivindicaciones referidas a una aplicación o un uso no son aceptables de conformidad con el Art. 14 D486.

- 5) Las reivindicaciones 11 y 85 no son claras puesto que se refiere a una pluralidad de ligandos que no está caracterizada.
- 6) La reivindicación 13 y sus dependientes encabezan como método pero se refieren a la caracterización de un compuesto asociado a un soporte sólido, es decir, el objeto que reclaman es un conjugado y no un método. En ese sentido, existe una falsa dependencia puesto que se está caracterizando el método en términos de una Markush cuando un método elegible para examen es una secuencia lógica de etapas, tal como se indicó anteriormente. El compuesto de fórmula I está asociado a un soporte sólido que no ha sido caracterizado en la reivindicación, pero de acuerdo con la descripción se trata de una esfera de poliestireno. Por otra parte, dada la amplitud del objeto reclamado al indicarse seis grupos R en la fórmula I, no se puede determinar el alcance de la misma. La expresión “*en donde R1 se selecciona de una cadena de aminoácidos rica en electrones Y*” no es clara.
- 7) Se sugiere restringir la reivindicación a aquellas realizaciones que fueron probadas teniendo en cuenta que no existe sustento para todas las opciones reclamadas:

Sustituyentes de acuerdo con la reivindicación 13 de la solicitud		Sustituyentes soportados en la descripción
R1	cadena lateral de aminoácidos rica en electrones Y	Etanotiol (Figura 13)
R2	H	H (Figura 13)
R3	Seleccionado de H, C1-C6alquilo, C1-C6alquiloSCH3; C0- C6alquiloC2-C6alquenilo, C0-C6alquilo C2-C6alquinilo, C1-C6 COOH, C1-C6alquiloOH, C1-C6alquiloN(R)2, C3-C8cicloalquilo, C1-C6alquilarilo, C1-C6alquilheteroarilo, C1-C6alquiloINC(O)C1-C6alquilo, C1-C6alquilcicloamida en donde cualquiera de los grupos arilo o heteroarilo pueden ser sustituidos independientemente con -OH, Cl, F, Br, -OCH3, SO3NH2 o -O-CH2-O-.	Diaminobutano (Figura 13)
R4	Seleccionado de H, C1-C6alquilo, C1-C6alquiloSCH3; C0- C6alquiloC2-C6alquenilo, C0-C6alquilo C2-C6alquinilo, C1-C6 COOH, C1-C6alquiloOH, C1-C6alquiloN(R)2, C3-C8cicloalquilo, C1-C6alquilarilo, C1-C6alquilheteroarilo, C1-C6alquiloINC(O)C1- C6alquilo, C1-C6alquilcicloamida en donde cualquiera de los grupos arilo o heteroarilo pueden ser sustituidos independientemente con -OH, Cl, F, Br, -OCH3, SO3NH2 o -O-CH2-O-.	Diaminobutano o Piperonilamina (Figura 13)
R5	Seleccionado de H, C1-C6alquilo, C1-C6alquiloSCH3; C0- C6alquiloC2-C6alquenilo, C0-C6alquilo C2-C6alquinilo, C1-C6 COOH, C1-C6alquiloOH, C1-C6alquiloN(R)2, C3-C8cicloalquilo, C1-C6alquilarilo, C1-C6alquilheteroarilo, C1-C6alquiloINC(O)C1-C6alquilo, C1-C6alquilcicloamida en donde cualquiera de los grupos arilo o heteroarilo pueden ser sustituidos independientemente con -OH, Cl, F, Br, -OCH3, SO3NH2 o -O-CH2-O-.	Diaminobutano o 4-(2-Aminoetil)bencenosulfonamina (figura 13)
R6	Seleccionado de H, C1-C6alquilo, C1-C6alquiloSCH3; C0- C6alquiloC2-C6alquenilo, C0-C6alquilo C2-C6alquinilo, C1-C6 COOH, C1-C6alquiloOH, C1-C6alquiloN(R)2, C3-C8cicloalquilo, C1-C6alquilarilo, C1-C6alquilheteroarilo, C1-C6alquiloINC(O)C1- C6alquilo, C1-C6alquilcicloamida en donde cualquiera de los grupos arilo o heteroarilo pueden ser sustituidos independientemente con -OH, Cl, F, Br, -OCH3, SO3NH2 o -O-CH2-O-.	Isobutilamina, metilbencilamina, metoxietilamina, cicloexilamina, 4-(2-Aminoetil)bencenosulfonamina o Piperonilamina (Figura 13).

- 8) La expresión “*en donde los compuestos se producen por un proceso que comprende el uso de un reactivo...*” no es válida ya que un compuesto debe definirse por sus características químicas, estructurales, biológicas o funcionales más no por su forma de producción (reivindicaciones 14 y 64).
- 9) En las reivindicaciones 15 y 16 se da a elegir entre múltiples opciones, las cuales no todas tienen sustento en la descripción. Se sugiere limitar la materia a aquellas realizaciones que fueron probadas.

- 10) La reivindicación 40 no revela alguna característica del método reclamado. Se refiere únicamente a que el método se realiza conforme las instrucciones de un prospecto, lo cual no es claro puesto que no está definido.
- 11) El término “ligandos MM” no es claro. Se sugiere hacer las aclaraciones necesarias.
- 12) Las reivindicaciones 79 y 72 corresponden a un resultado a alcanzar, se sugiere remplazar por las características propias del método que permita llegar a dichos resultados.

Descripción (Art. 28 D486)

No existe sustento en la descripción para todas las modalidades reclamadas en las reivindicaciones. Se sugiere modificar las reivindicaciones para que sean consistentes con la materia divulgada.

7. Unidad de invención (Art 25 D 486)

La solicitud no tiene unidad de invención *a priori* puesto que el objeto está reclamado de acuerdo a una estructura Markush donde se da a escoger entre múltiples opciones para cada radical reclamado en la fórmula I, donde lo único fijo es el grupo amino. Como puede observarse en la reivindicación 13, el radical R1 puede ser cualquier aminoácido y los radicales R3 – R6 pueden seleccionarse entre trece sustituyentes los cuales pueden presentar hasta siete sustituciones en cada uno de los grupos funcionales, de modo que no existen características técnicas esenciales que definan el objeto a reclamar y lo diferencien del estado de la técnica.

Por consiguiente, el alcance del objeto reclamado es tan amplio que no es posible reconocer un único concepto común inventivo.

Se le sugiere al solicitante restringir la materia a patentar en un solo concepto común inventivo especificando el avance tecnológico sobre el estado de la técnica.

8. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: marzo 24 de 2011, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	US20090246124	“High affinity vegf-receptor antagonists”	01/Octubre/2009
D2	Reddy & Kodadek 2005	“Protein “fingerprinting” in complex mixtures with peptoid microarrays”	06/Septiembre/2005

El documento D1¹ divulga un método para identificar compuestos de unión a marcadores celulares en una biblioteca combinatoria de peptoides (oligómeros de N-alquilglicina) por medio de microesferas ancladas a una matriz (Resumen).

El documento D2² divulga el empleo de matrices compuestas de una librería de peptoides para identificar proteínas en muestras biológicas (Pág. 12672).

9. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La reivindicación 1 consiste en “Un método para diagnosticar una respuesta inducida por fármaco

¹ <http://www.google.com/patents/US20090246124>

² Reddy & Kodadek 2005. Protein “fingerprinting” in complex mixtures with peptoid microarrays. PNAS 102(36): 12672-12677. Expediente 13-250606- -5 1er Art 45

en un sujeto, el método que comprende: la obtención de una muestra biológica de dicho sujeto; la selección de una genoteca de ligandos contra dicha muestra...”

La reivindicación 47 consiste en “Un método para el perfilado de uno o más sujetos para una respuesta de un fármaco...”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Un método para diagnosticar una respuesta inducida por fármaco en un sujeto	Método (Párr. [0110])
obtención de una muestra biológica de dicho sujeto	Obtener muestra biológica de un paciente (Párr.[0008])
selección de una genoteca de ligandos contra dicha muestra	Contactar la muestra con un peptoide (Párr.[0027])
identificación de las características de unión de uno o más marcadores en dicha muestra con uno o varios ligandos en la genoteca	Evaluar la unión entre ligando y marcador (Párr.[0030])
determinar si dicho uno o más marcadores están asociados con dicha respuesta inducida por fármacos, diagnosticando de tal modo dicha respuesta inducida por fármacos en dicho sujeto	Determinar si dicho ligando es capaz de actuar como antagonista del marcador Párr.[0102])

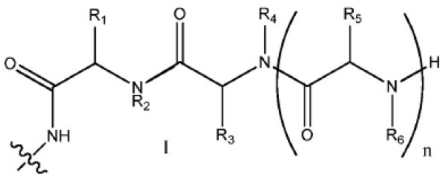
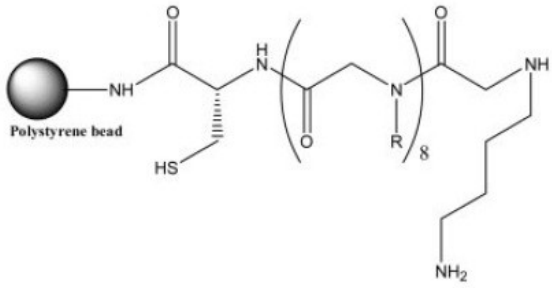
Las características esenciales de los métodos de las reivindicaciones 1 y 47 habían sido divulgadas previamente en el documento D1, el cual divulga un método de identificación de compuestos de una librería de peptoides asociadas a microesferas que se unen específicamente a un marcador en células de un sujeto (Resumen). Dicho método consiste en contactar las células con peptoides y evaluar la unión entre ligando y marcador para de ese modo determinar si dicho ligando es capaz de actuar como antagonista del marcador identificado en la muestra (Párr. [0027-0030]).

En consecuencia, la reivindicación 1 no es nueva, según lo divulgado en el documento D1. Las reivindicaciones dependientes 2 – 12, 17 – 42, 46, no mencionan alguna característica que haga nuevo al método de la reivindicación 1, por lo cual se considera que no son nuevas.

La reivindicación 47 tampoco es nueva, según lo divulgado en el documento D1. Las reivindicaciones dependientes 48 – 49, 52 – 62, 67 – 96 no mencionan alguna característica que haga nuevo al método de la reivindicación 47, por lo cual se considera que no son nuevas.

La reivindicación 13 consiste en: “El método de conformidad con la reivindicación 1, caracterizado porque la genoteca comprende un compuesto de fórmula I, en donde R1 se selecciona de una cadena lateral de aminoácidos...”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Genoteca que comprende un compuesto de fórmula I 	Librería de peptoides de estructura general (figura 1): 
En donde	En donde
R1 se selecciona de cadena lateral de	R1 se selecciona de cadena lateral de aminoácidos rica

aminoácidos rica en electrones Y	en electrones (Figura 1)
R2 es H	R2 es H (figura 1)
R3 es seleccionado de H, C1-C6alquilo, C1-C6alquiloSCH3; C0- C6alquiloC2-C6alquenilo, C0-C6alquilo C2-C6alquinilo, C1-C6COOH, C1-C6alquiloOH, C1-C6alquiloN(R)2, C3-C8cicloalquilo, C1-C6alquilarilo, C1-C6alquilheteroarilo, C1-C6alquiloINC(O)C1-C6alquilo, C1-C6alquilocicloamida en donde cualquiera de los grupos arilo o heteroarilo pueden ser sustituidos independientemente con -OH, Cl, F, Br, -OCH3, SO3NH2 o -O-CH2-O-.	Aminas seleccionadas de etanolamina, piperonilamina, glicina, metilbencilamina, diaminobutano, isobutilamina y alilamina (Figura 1).
R4 Seleccionado de H, C1-C6alquilo, C1-C6alquiloSCH3; C0- C6alquiloC2-C6alquenilo, C0-C6alquilo C2-C6alquinilo, C1-C6COOH, C1-C6alquiloOH, C1-C6alquiloN(R)2, C3-C8cicloalquilo, C1-C6alquilarilo, C1-C6alquilheteroarilo, C1-C6alquiloINC(O)C1-C6alquilo, C1-C6alquilocicloamida en donde cualquiera de los grupos arilo o heteroarilo pueden ser sustituidos independientemente con -OH, Cl, F, Br, -OCH3, SO3NH2 o -O-CH2-O-.	
R5 Seleccionado de H, C1-C6alquilo, C1-C6alquiloSCH3; C0- C6alquiloC2-C6alquenilo, C0-C6alquilo C2-C6alquinilo, C1-C6COOH, C1-C6alquiloOH, C1-C6alquiloN(R)2, C3-C8cicloalquilo, C1-C6alquilarilo, C1-C6alquilheteroarilo, C1-C6alquiloINC(O)C1-C6alquilo, C1-C6alquilocicloamida en donde cualquiera de los grupos arilo o heteroarilo pueden ser sustituidos independientemente con -OH, Cl, F, Br, -OCH3, SO3NH2 o -O-CH2-O-.	
R6 Seleccionado de H, C1-C6alquilo, C1-C6alquiloSCH3; C0- C6alquiloC2-C6alquenilo, C0-C6alquilo C2-C6alquinilo, C1-C6COOH, C1-C6alquiloOH, C1-C6alquiloN(R)2, C3-C8cicloalquilo, C1-C6alquilarilo, C1-C6alquilheteroarilo, C1-C6alquiloINC(O)C1-C6alquilo, C1-C6alquilocicloamida en donde cualquiera de los grupos arilo o heteroarilo pueden ser sustituidos independientemente con -OH, Cl, F, Br, -OCH3, SO3NH2 o -O-CH2-O-.	

Las características esenciales de los compuestos reclamados en la reivindicación 13 habían sido divulgadas previamente en el documento D2, el cual divulga un método de identificación de compuestos de una librería de peptoides asociadas a esferas de poliestireno. La librería de peptoides tiene una estructura general como la representada en la figura 1, donde los radicales pueden ser seleccionados de etanolamina, piperonilamina, glicina, metilbencilamina, diaminobutano, isobutilamina y alilamina (Pág. 12673, figura 1).

En consecuencia, la reivindicación 13 no es nueva, según lo divulgado en el documento D2. Las reivindicaciones dependientes 13 - 16, no mencionan alguna característica que haga nuevo al compuesto de la reivindicación 13, por lo cual se considera que no son nuevas.

10. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US20090246124	1 – 12, 17 – 39, 46 – 49, 52 – 62, 67 – 96	Novedad
D2	Reddy & Kodadek 2005	13 – 16	Novedad

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



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Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-03-17

Elaboró: Edna Carolina Bonilla Leon

Revisó: Consuelo Leguizamon Leguizamon

Protein “fingerprinting” in complex mixtures with peptoid microarrays

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Edited by Jack W. Szostak, Harvard University, Boston, MA, and approved July 25, 2005 (received for review February 15, 2005)

We report here that microarrays comprised of several thousand peptoids (oligo-*N*-substituted glycines) are useful tools for the identification of proteins via a “fingerprinting” approach. By using maltose-binding protein, glutathione *S*-transferase, and ubiquitin, a specific and highly reproducible pattern of binding was observed when fluorescently labeled protein was hybridized to the array. A similar pattern was obtained when binding of an unlabeled protein to the array was visualized by secondary hybridization of a labeled antibody against that protein, showing that native proteins can be identified without the requirement for prior chemical labeling. This work suggests that small-molecule microarrays might be used for more complex fingerprinting assays of potential diagnostic value.

profiling | proteomics | small molecule microarrays

Small-molecule microarrays (SMMs) (1–3) are becoming increasingly important tools in combinatorial chemistry. These arrays are generally produced by first synthesizing a combinatorial library on a suitable bead resin, separating the beads into the wells of microtiter plates, and then releasing the compounds from the beads (4, 5). The resulting solutions then are spotted robotically onto a chemically modified glass slide such that the library-derived molecule is attached covalently to the surface. Alternatively, methods exist for the synthesis of certain classes of compounds *in situ* on the array surface (6–10).

By far the most common application of SMMs has been as a versatile platform for library screening, usually with the goal of identifying small-molecule ligands for a given protein of interest (1, 11–13). However, little work has focused on the development of SMMs as analytical tools for biological research (14–16), with the notable exception of peptide arrays as tools for determining the substrate preferences for proteases and other protein-modifying enzymes (12, 17–21) and the binding preferences of antibodies (22–24). We hypothesized that hybridization of any particular protein to a SMM with thousands of features is likely to provide a unique pattern of binding to the array, allowing that factor to be identified by virtue of this “molecular fingerprint.” Indeed, screening experiments have shown qualitatively that different high-affinity ligands are usually, although not always, identified when different proteins are incubated with a combinatorial library (1). The fingerprinting idea simply extends this concept to the quantitative measurement of protein binding at most or all features on a SMM. The expectation is that whereas only a few molecules in a library will be high-affinity ligands for a given protein, a larger number would exhibit above-background binding with a broad spectrum of affinities. Quantitation therefore would provide a fingerprint unique to that protein, because it seems exceedingly unlikely that any two proteins would bind to thousands of different compounds with similar affinities.

Although this approach to protein identification seems reasonable, a number of important technical issues must be addressed to determine its feasibility. For example, libraries that are either large and/or rich in general protein-binding compounds must be made and arrayed, because the fingerprinting concept would not work if only a few compounds on an array

bound the target protein above background. Another critical issue is the reproducibility of this sort of experiment. Derivation of a fingerprint will be far more demanding in this regard than identifying a few of the brightest spots on the array, as is the case in ligand discovery screens.

We show here that arrays comprised of several thousand peptoids (oligo-*N*-substituted glycines) (25–28) can indeed be used to measure protein fingerprints in a highly reproducible fashion. We also demonstrate that this approach can be combined with an antibody-based “sandwich assay” to identify proteins in complex mixtures without chemical labeling of the sample. These studies suggest that peptoid-based SMMs could be developed as useful tools for protein identification in biological milieu.

Materials and Methods

General Remarks. All chemicals and solvents were purchased from commercial suppliers and used without further purification. Ubiquitin (Ub) was obtained from Sigma–Aldrich, maltose-binding protein (MBP) was from New England Biolabs, goat anti-mouse IgG and Alexa Fluor 488 were obtained from Molecular Probes, and anti-glutathione *S*-transferase (GST) antibody was from Santa Cruz Biotechnology. All proteins were labeled by using standard protocols (27, 29). The slides were scanned by using a ScanArray ExpressHT Microarray Scanner (PerkinElmer) at 10- μ m resolution with 488- and 532-nm excitation lasers.

Peptoid Library. The library used in this study was synthesized by using the “submonomer” method (30) as described in ref. 27, except that microwave irradiation (31) was used to accelerate all of the synthetic steps.

Preparation of Peptoid Microarrays. Peptoid stock solutions were printed onto a chemically functionalized (maleimide) glass slides by using SpotArray 72 Microarray Printing System (PerkinElmer). The slides then were allowed to stand for 15 h on the printer platform and washed 1 h each with DMSO, dimethylformamide, tetrahydrofuran, and isopropanol. Slides were dried by centrifugation and stored under argon at room temperature. Full details of slide preparation are available from the authors on request.

Microarray Hybridization and Image Analysis. Peptoid microarrays were scanned and hybridized with various proteins as described in ref. 29. Slides were equilibrated with 1 $\times$ TBST (50 mM Tris/150 mM NaCl/0.1% Tween 20, pH 7.4) for 15 min and blocked with *Escherichia coli* lysate for 1 h at 4°C. After each step, microarray slides were rinsed in 1 $\times$ TBST before applying

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Abbreviations: MBP, maltose-binding protein; SMM, small-molecule microarray; Ub, ubiquitin.

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the next protein. Each protein was diluted (see text for concentrations) with 1× TBST containing 100-fold excess of *E. coli* lysate and applied to the slides. Microarray slides then were incubated for 2 h at 4°C with gentle shaking. The slides were rinsed once and washed with 1× TBST (3 × 4 min), then dried by centrifugation.

Microarrays were scanned with a ScanArray ExpressHT Microarray Scanner using blue 488-nm (fluorescein-labeled proteins) and green 543-nm (Cy3-labeled proteins) lasers at 100% power and 70% photomultiplier tube gain. All of the scanned images were analyzed by using the GENEPIX PRO 5.0 software (Axon Instruments, Union City, CA). Local background subtracted mean spot intensities were used for further analysis. All of the spot intensities of a slide before protein hybridization were subtracted from the same slide after protein hybridization in EXCEL to get the true signal intensity due to protein binding to each feature. These true signal intensities were used for downstream analysis with GENESPRING software (Silicon Genetics, Palo Alto, CA). Only features that gave positive signal intensity values on both experiments were used to determine reproducibility and standard correlation values. We used these intensities rather than a ratio in which the intensity at any given spot is referenced to that obtained at a control feature. This approach, which is used routinely in DNA microarray analysis, is problematic in this case. In a DNA microarray, or any array in which each feature is designed to capture a specific analyte, one can design controls that are not expected to bind anything specifically. In a protein-fingerprinting experiment, it is not possible to do so.

Results

Different Proteins Exhibit Unique and Reproducible Fingerprints When Hybridized to a Peptoid Microarray. We constructed microarrays consisting of 7,680 different octameric peptoids spotted covalently on a maleimide-functionalized glass microscope slide by using a robotic pin spotter. The peptoid library was created by split and pool synthesis on 500-μm polystyrene macrobeads (Rapp Polymere, Tübingen, Germany) by using the amines shown in Fig. 1. A C-terminal cysteine residue was included in each molecule to facilitate coupling to the array surface.

To these arrays was hybridized either fluorescein-labeled Ub, fluorescein-labeled GST, or Cy3-labeled MBP in the presence of a 100-fold excess of unlabeled proteins derived from a crude *E. coli* extract (to mimic a moderately abundant protein in a crude extract). The concentration of the labeled protein was 500 nM in each case. After washing, the pattern of binding of the labeled protein to the array was visualized by using a standard commercial microarray scanner. Each experiment was done twice in a completely independent fashion. The raw array images from the first set of hybridizations are shown in Fig. 2, along with an image of an array taken before protein hybridization (far left). The visible spots on this control array represent fluorescein-containing marker peptoids spotted as navigation aids. Hundreds of features on each array captured labeled protein at a level detectable above the background under these conditions, as can be better seen in the expanded regions shown in Fig. 2. These data confirm that a library of 7,680 peptoids is sufficiently rich in protein ligands to support a fingerprinting application.

Even with the naked eye, it can be seen that the binding patterns are distinct on each array (Fig. 2). To better illustrate and quantify this distinction, the fluorescent intensity at each feature (from 1 to 7,680) was quantified and, after subtraction of the background intensity (see *Materials and Methods*), was displayed as a color-coded bar graph with each line representing the background-subtracted intensity of a single feature using the GENESPRING software package (Fig. 3). Examination of these bar codes revealed that each protein provided a unique pattern of binding to the array, although, surprisingly, the Ub and GST data

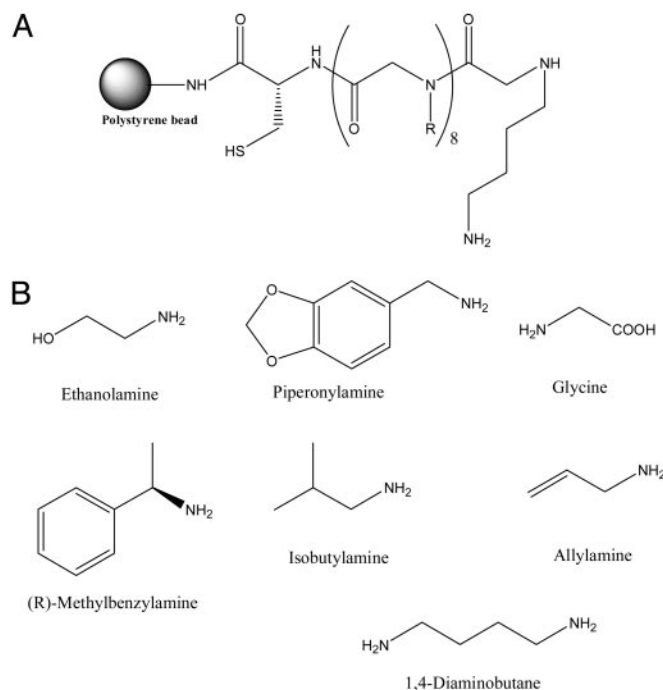


Fig. 1. The peptoid library used in this study. (A) General structure of the peptoid library synthesized on polystyrene macrobeads. (B) Amines used in the synthesis of the library.

sets superficially resembled one another, whereas that produced in the MBP hybridization was quite different. Focusing on almost any smaller set of features clearly illustrates the differences between the Ub and GST binding patterns (Fig. 3, bottom).

To address the critical issue of reproducibility and the uniqueness of the fingerprint in another way, all of the data were visualized as a series of scatter plots. When the two independent hybridizations for a given protein were compared, a high degree of correlation was obtained (Fig. 4A) ($R = 0.97$ for GST, 0.96 for MBP, and 0.97 for Ub). An even better correlation was obtained if only the higher-intensity features were considered, as one would expect, because the data closest to the background tend to be the “noisiest” (see Fig. 6, which is published as supporting information on the PNAS web site) ($R = 0.99$ for all proteins). To better display how many peptoids bind promiscuously to all of the proteins and how many are specific, all of the features that display an intensity value of ≥ 10 -fold above background were incorporated into a Venn diagram (see Fig. 7, which is published as supporting information on the PNAS web site); 191, 126, and 61 peptoids were found to be quite specific for GST, MBP, and Ub, respectively. We conclude that the technique is sufficiently reproducible and has a high enough degree of specificity to serve as a platform for protein fingerprinting.

When the data sets obtained from two different protein hybridizations were compared, the correlation was far lower (see Fig. 4B) ($R = 0.56$ for GST vs. Ub, 0.28 for GST vs. MBP, and 0.25 for Ub vs. MBP). Based on these data, we conclude that the three different proteins used in this study exhibit highly reproducible and distinctive patterns when hybridized to the peptoid microarray.

An Antibody Sandwich Assay Allows Detection of the Binding Pattern of an Unlabeled Protein. The proof of principle experiments presented above used purified proteins that had been labeled covalently and then mixed with the bacterial extract. Obviously, this type of protocol could not have been used to detect a native

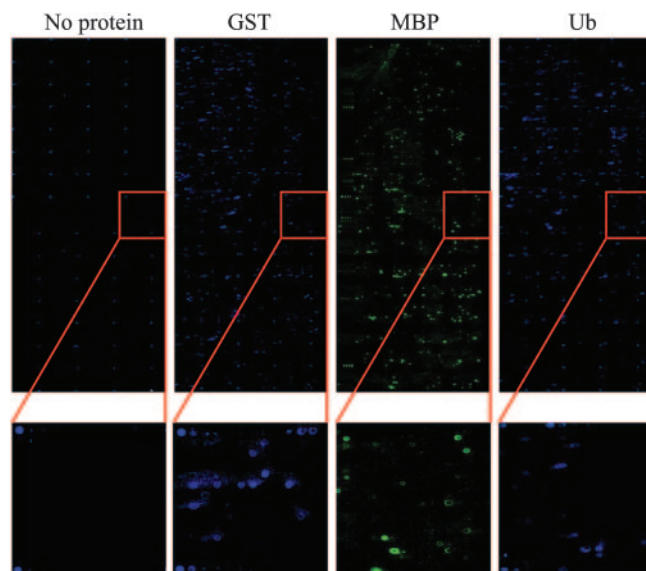


Fig. 2. Protein profiling using peptoid microarrays. Images were obtained by incubating fluorescently labeled GST, MBP, or Ub to a peptoid microarray containing 7,680 different compounds. For comparison, a fourth array to which no protein has been hybridized is shown on the far left. In this image, the fluorescent spots are labeled peptoids that have been attached to the slide as navigation aids. These images were obtained by scanning the arrays with a standard commercial array scanner used for DNA microarray analysis after hybridization and washing. The insets provide a magnified view to illustrate the signal-to-noise ratio obtained in the hybridization of 500 nM labeled protein to the array.

protein. An alternative method that would achieve this goal would be to hybridize to the array a mixture of native proteins, then probe the chip with an antibody raised against a particular protein of interest, followed by a labeled secondary antibody (13). Therefore, we performed an experiment in which unlabeled GST was doped into a 100-fold excess of bacterial proteins and hybridized to the array. To visualize the GST binding pattern selectively, the array was then probed with an anti-GST antibody followed by a secondary antibody labeled with Alexa Fluor 488. As a control, a second array was treated in the same way, except that the GST was omitted. The fluorescent intensities at each feature then were measured, and the values obtained in the “–GST” experiment were subtracted from those in the “+GST” experiment to provide a corrected data set from which the binding patterns of the antibodies had been subtracted. Again, two completely independent experiments were conducted, and these experiments were highly reproducible (Fig. 5A) ($R = 0.96$). A comparison of this data set with that obtained with the chemically labeled GST (Fig. 5B) revealed a similar, but not identical, protein fingerprint. The correlation coefficient was $R = 0.84$. Although errors introduced in the subtraction of the “antibody-only” control data could play some role in this reduced correlation, the similarity of this scatter plot to those generated by using data sets from the hybridization of different proteins (Fig. 4B) suggests an alternative explanation. The dramatically “off-diagonal” features clearly cluster into two groups, one of which registers a much higher signal intensity in the antibody-using experiment whereas the other provides a much higher signal in the experiment by using chemically labeled GST. This result suggests that the features in these two groups discriminate significantly between the labeled and native forms of the GST. In other words, the simplest explanation of the data are that native and fluorescein-labeled GST appear to the array as related, but different, proteins, although detailed binding studies of some of these peptoids to each form of the protein will

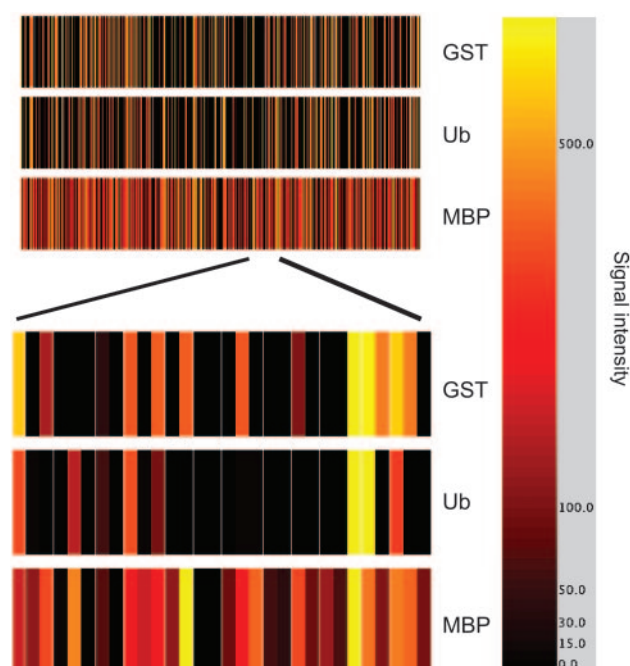


Fig. 3. A protein fingerprint. The intensities at each of the 7,680 features on the arrays shown in Fig. 2 (and the replicate experiments) were assigned a color code (shown on the right) and displayed as a bar code to allow visualization of the binding patterns. A portion of this bar code is expanded to show clearly the differences between the binding patterns.

be required to substantiate this hypothesis. In any case, this experiment shows clearly that a distinctive fingerprint of a native protein can be detected on the peptoid microarray by using an antibody-based sandwich assay.

Sensitivity of the Peptoid Microarrays. The experiments discussed above used the target protein at a concentration of 500 nM in the presence of a 100-fold excess of bacterial proteins. To determine whether more dilute proteins could be detected and fingerprinted by using the peptoid microarray, fluorescein-labeled GST solutions of 100 and 10 nM, again in a solution containing a 100-fold excess of bacterial proteins, were prepared and hybridized to the peptoid arrays. After washing, the arrays were scanned, and the intensity observed at each feature was quantified as described above. The data then were compared. This comparison revealed an excellent correlation (see Fig. 8, which is published as supporting information on the PNAS web site) between the data sets obtained at the different GST concentrations, with correlation coefficients of $R = 0.95$, $R = 0.97$, and $R = 0.95$ for comparison of the 500 vs. 100 nM, 100 vs. 10 nM, and 500 vs. 10 nM data sets, respectively. In other words, very similar protein fingerprints could be discerned at each of the protein concentrations examined. In another experiment, GST (500 nM) was serially diluted into an *E. coli* lysate to create solutions with a 500-fold (GST = 0.5% of total protein) or 1,000-fold (GST = 0.1% of total protein) excess of bacterial proteins. After hybridization to the arrays and detection as described above, the data sets were compared on a scatter plot (see Fig. 9, which is published as supporting information on the PNAS web site). The R values derived from these scatter plots were 0.96 for 100-fold vs. 500-fold, 0.97 for 100-fold vs. 1,000-fold, and 0.97 for 500 vs. 1,000-fold. In other words, these data show that a recognizable and consistent fingerprint is observed for a protein present in an extract at different levels and is not sensitive to the abundance of that protein. Another issue that can be addressed with these

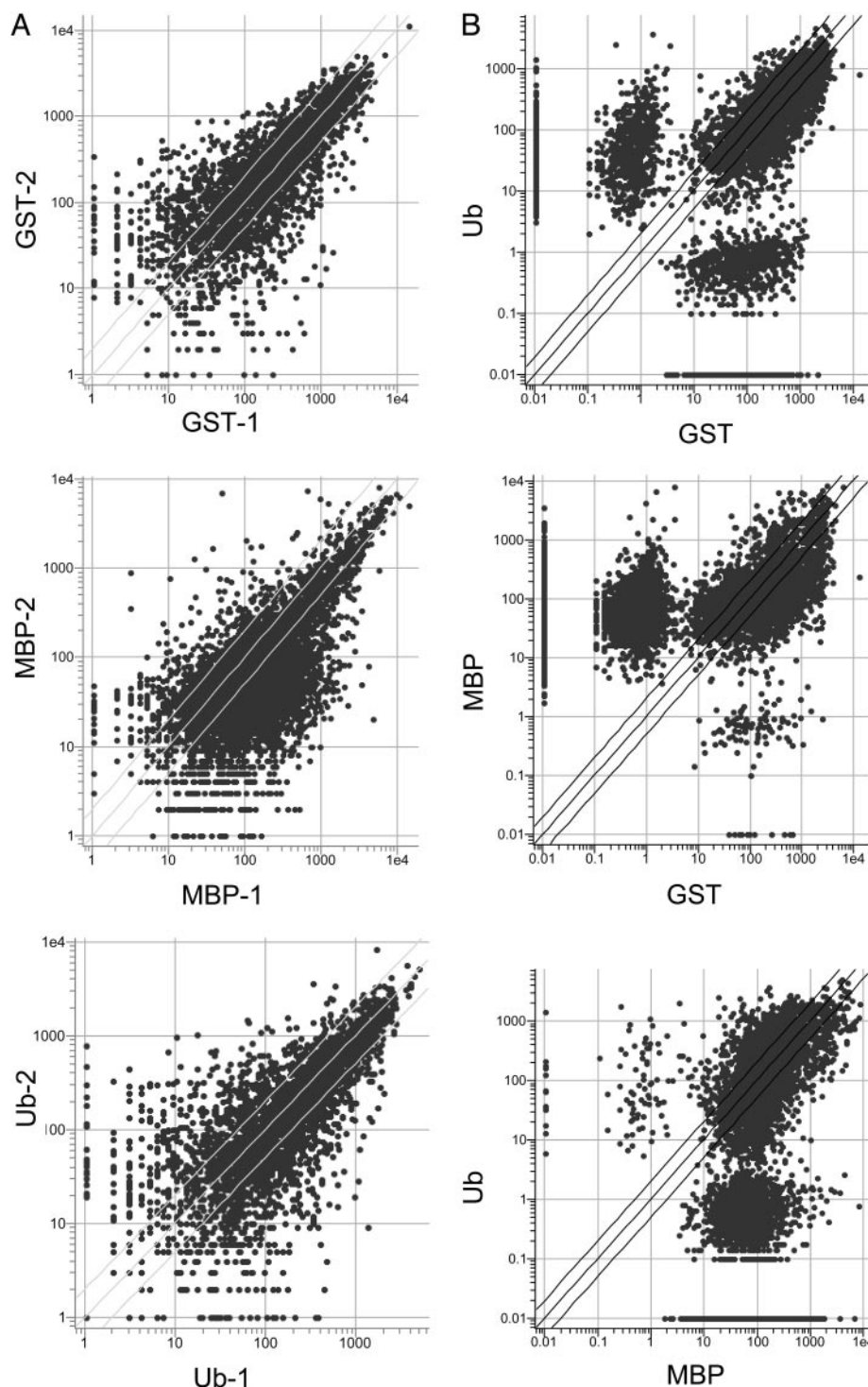


Fig. 4. Reproducibility and the degree of uniqueness of the protein fingerprint. (A) Scatter plots comparing the data obtained in two completely independent experiments employing the same protein. High correlation coefficients were obtained (see text). (B) Scatter plots comparing the data obtained in the three different protein hybridization experiments, showing the large number of off-diagonal spots, which represent features that discriminate between the two proteins. Much lower correlation coefficients were obtained (see text).

8 data sets is whether the signal intensities on the arrays accurately reflected the absolute concentration of the protein. To address this point, the signal intensities for all of the features that displayed above-background binding in the 500 and 100 nM GST data sets were placed on a scatter plot (see Fig. 8). The slope of the best-fit line through these data was ≈ 5.7 . When only the 100 most intense spots were considered, the average intensity dif-

ference between the two experiments was 5.2-fold (data not shown). Thus, the array results reflect the protein concentration in these two experiments. However, when the same type of analyses were repeated for the 100- and 10-nM GST data sets, the average difference was only ≈ 3 -fold rather than the 10-fold expected ideally. In other words, the intensities in the 10-nM data set compared with 100-nM data set did not drop off as much

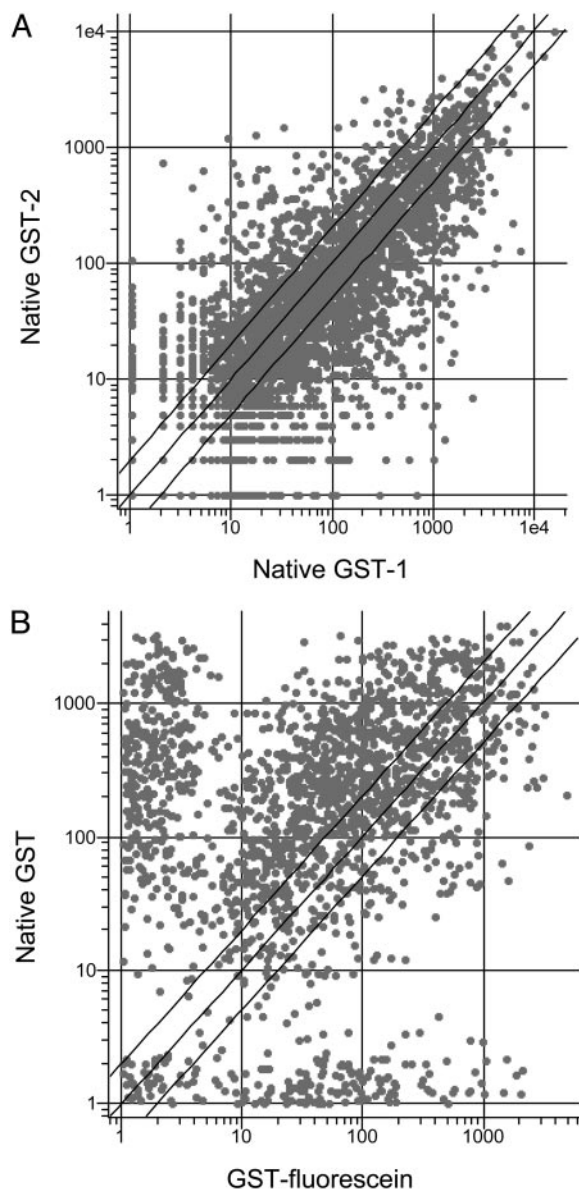


Fig. 5. Fingerprinting native GST by using an antibody sandwich assay (see text for details). (A) Scatter plot comparing the two completely independent experiments using native GST. (B) Scatter plot comparing the data obtained with native GST and fluorescein-labeled GST (Fig. 2).

as one would expect based on the absolute protein concentration. These data suggest that we are approaching the sensitivity limit of the array somewhere between 100 and 10 nM fluorescein-labeled GST.

Discussion

Both biological (32) and artificial (33, 34) sensors containing a large number of low to modest affinity receptors can differentiate between different molecules by distinguishing quantitative patterns of binding of an analyte to the different receptors. We have combined this concept with peptoid microarrays to create a powerful platform for the identification of proteins. Proof of principle experiments using chemically labeled proteins (Figs. 2–4) demonstrated that different proteins exhibit a different pattern of binding to the thousands of peptoids on the array. We also demonstrated that a protein fingerprint could be discerned when a native protein (GST) in a solution containing a large

excess of bacterial proteins was hybridized to the array (Fig. 5). Selective visualization of the GST binding pattern even in the presence of a large excess of other proteins was achieved by conducting a second hybridization with an anti-GST antibody. The pattern due to antibody binding was determined in a control experiment and subtracted from these data. This finding is critical in further development of these arrays as bioanalytical tools, because selective chemical labeling of a protein of interest in a complex mixture can rarely be achieved. A small amount of analytical work has been done to distinguish purified proteins with simpler arrays in other configurations (14–16). However, this work provides a previously undescribed demonstration that a glass slide-based SMM can “fingerprint” a native protein in a complex mixture. Because the proteins used in this study, GST, MBP, and Ub, are not related to one another and because the molecules in the library used to make the array (Fig. 1) were not biased in any way, we presume that the peptoid microarray is capable of fingerprinting almost any protein, although substantiation of this hypothesis obviously will require further study.

This array-based fingerprinting technique proved to be fairly sensitive, with a recognizable fingerprint produced by using a GST solution of only 10 nM in the presence of a 100-fold excess of bacterial proteins (Fig. 8). This issue was a major question to be addressed going into this study, because none of the peptoids were anticipated to be high-affinity ligands for GST or any other protein. Our experience with peptoid library screening suggests that even the best ligands in an unbiased library are likely to form complexes with K_D values in the low-micromolar range (27). However, the linearity of the signal intensities deviated from the expected values between 100 and 10 nM for labeled GST, indicating that the sensitivity limit for truly quantitative work was somewhere between 100 and 10 nM GST in this particular case. Nonetheless, this limit would be sufficient to fingerprint many highly and modestly abundant proteins in serum or cell extracts. Furthermore, this limit is not necessarily a general sensitivity limit of the array, because this limit will depend on the affinities of the protein analyzed for the peptoids on the array. For example, we have evidence that antibodies and certain other proteins bind with unusually high affinity to many peptoids (M.M.R. and T.K., unpublished results) on the microarray. It is also possible that much higher sensitivity could be achieved by using more intensely fluorescent dyes such as quantum dots or with detection schemes that employ some sort of signal amplification.

The experiments reported here are model studies and break no new biological ground. The presence and levels of a protein in a complex mixture could have been determined with SDS/PAGE and Western blotting. Nonetheless, these experiments demonstrate two critical points that were far from obvious before these studies. The first is that the patterns obtained are highly reproducible (Fig. 4) in completely independent experiments. The second is that even an array with a relatively modest number of features (<8,000) is capable of a high level of discrimination between two proteins. In other words, the collection of peptoids is sufficiently rich in specific ligands for any given protein that a unique pattern is produced. Indeed, it was quite interesting that a comparison between the binding patterns obtained with native GST and fluorescein-labeled GST were noticeably different. This finding suggests that the distinguishing power of the array may be sufficient to derive different patterns for the same protein with different posttranslational modifications. This application would be an attractive use of these arrays, because antibodies that recognize specific forms of a given protein are often difficult to obtain. To read the array pattern, only a standard antibody that recognized any form of the protein would be necessary.

Another potential application of this array technology is protein profiling for diagnostic purposes. By using other analytical platforms, particularly surface-enhanced laser desorption/ionization mass spectrometry, several investigators have argued

that protein profiling of complex samples can be used to diagnose cancers and other disease states (for reviews, see refs. 35–37). This application is a different kind of fingerprint or profiling experiment than we have reported here using SMM array technology. In Protein Chip/surface-enhanced laser desorption ionization experiments, the subset of the serum proteome that binds to the surfaces on the chip and that are ionized efficiently in the mass spectrometer is analyzed to produce a pattern of peaks that is taken as a potential fingerprint of a disease state. This technique thus looks at a large number of different proteins whose identities are unknown. Although we have focused on binding patterns of single proteins in this study, one could easily imagine examination of binding “superpatterns” comprised of the superposition of many individual protein-binding events. Such an experiment would use a visualization reagent that would recognize many different proteins, such as an anti-phosphotyrosine antibody that would “light up” any protein

with this modification. Another possibility would be to label certain classes of enzymes, such as serine proteases, with activity-based labeling reagents before hybridization (38). The high level of reproducibility observed in the simpler experiments reported here suggests that the peptoid array technology would be highly competitive with surface-enhanced laser desorption ionization for more complex profiling applications.

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- Uttamchandani, M., Walsh, D. P., Yao, S. Q. & Chang, Y.-T. (2005) *Curr. Opin. Chem. Biol.* **9**, 4–13.
- MacBeath, G., Koehler, A. N. & Schreiber, S. L. (1999) *J. Am. Chem. Soc.* **121**, 7967–7968.
- Lam, K. S. & Renil, M. (2002) *Curr. Opin. Chem. Biol.* **6**, 353–358.
- Blackwell, H. E., Perez, L., Stavenger, R. A., Tallarico, J. A., Eatough, E. C., Foley, M. A. & Schreiber, S. L. (2001) *Chem. Biol.* **8**, 1167–1182.
- Clemons, P. A., Koehler, A. N., Wagner, B. K., Springings, T. G., Spring, D. R., King, R. W., Schreiber, S. L. & Foley, M. A. (2001) *Chem. Biol.* **8**, 1183–1195.
- Fodor, S. P., Read, J. L., Pirrung, M. C., Stryer, L., Lu, A. T. & Solas, D. (1991) *Science* **251**, 767–773.
- Frank, R. (2002) *J. Immunol. Methods* **267**, 13–26.
- Li, S., Bowerman, D. J., Marthandan, N., Garner, H. R. & Kodadek, T. (2004) *J. Am. Chem. Soc.* **126**, 4088–4089.
- Shaginan, A., Patel, M., Li, M.-H., Flickinger, S. T., Kim, C., Cerrina, F. & Belshaw, P. J. (2004) *J. Am. Chem. Soc.* **126**, 16704–16705.
- Li, S., Marthandan, N., Bowerman, D., Garner, H. R. & Kodadek, T. (2005) *Chem. Commun.*, 581–583.
- Kuruvilla, F. G., Shamji, A. F., Sternson, S. M., Hergenrother, P. J. & Schreiber, S. L. (2002) *Nature* **416**, 653–657.
- Winssinger, N., Ficarro, S., Schultz, P. G. & Harris, J. L. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 11139–11144.
- Koehler, A. N., Shamji, A. F. & Schreiber, S. L. (2003) *J. Am. Chem. Soc.* **125**, 8420–8421.
- Takahashi, M., Nokihara, K. & Mihara, H. (2003) *Chem. Biol.* **10**, 53–60.
- Usui, K., Ojima, T., Takahashi, M., Nokihara, K. & Mihara, H. (2004) *Biopolymers (Pept. Sci.)* **76**, 129–139.
- Baldini, L., Wilson, A. J. & Hamilton, A. D. (2004) *J. Am. Chem. Soc.* **126**, 5656–5657.
- Harris, J. L., Alper, P. B., Li, J., Rechsteiner, M. & Backes, B. J. (2001) *Chem. Biol.* **8**, 1131–1141.
- Mason, D. E., Ek, J., Peters, E. C. & Harris, J. L. (2004) *Biochemistry* **43**, 6535–6544.
- Martin, K., Steinberg, T. H., Cooley, L. A., Gee, K. R., Beechem, J. M. & Patton, W. F. (2004) *Proteomics* **3**, 1244–1255.
- Falsey, J. R., Renil, M., Park, S.-H., Li, S. & Lam, K. S. (2001) *Bioconj. Chem.* **12**, 346–353.
- Lesacherre, M.-L., Mahesh, U., Chen, G. Y. J. & Yao, S. Q. (2002) *Bioorg. Med. Chem. Lett.* **12**, 2085–2088.
- Jellis, C. L., Cradick, T. J., Rennert, P., Salinas, P., Boyd, J., Amirault, T. & Gray, G. S. (1993) *Gene* **137**, 63–68.
- Beattie, J., Shand, J. H. & Flint, D. J. (1996) *Eur. J. Biochem.* **239**, 479–486.
- Reineke, U., Ivascu, C., Schlieff, M., Landgraf, C., Gericke, S., Zahn, G., Herzel, H., Volkmer-Engert, R. & Schneider-Mergener, J. (2002) *J. Immun. Methods* **267**, 37–51.
- Simon, R. J., Kania, R. S., Zuckermann, R. N., Huebner, V. D., Jewell, D. A., Banville, S., Ng, S., Wang, L., Rosenberg, S., Marlowe, C. K., *et al.* (1992) *Proc. Natl. Acad. Sci. USA* **89**, 9367–9371.
- Figliozzi, G. M., Goldsmith, R., Ng, S. C., Banville, S. C. & Zuckermann, R. N. (1996) *Methods Enzymol.* **267**, 437–447.
- Alluri, P. G., Reddy, M. M., Bachawat-Sikder, K., Olivos, H. J. & Kodadek, T. (2003) *J. Am. Chem. Soc.* **125**, 13995–14004.
- Heine, N., Ast, T., Schneider-Mergener, J., Reineke, U., Germeroth, L. & Wenschuh, H. (2003) *Tetrahedron* **59**, 9919–9930.
- Reddy, M. M., Bachawat-Sikder, K. & Kodadek, T. (2004) *Chem. Biol.* **11**, 1127–1137.
- Zuckermann, R. N., Kerr, J. M., Kent, S. B. H. & Moos, W. H. (1992) *J. Am. Chem. Soc.* **114**, 10646–10647.
- Olivos, H. J., Alluri, P. G., Reddy, M. M., Saloney, D. & Kodadek, T. (2002) *Org. Lett.* **4**, 4057–4059.
- Axel, R. (1996) *Cold Spring Harbor Symp. Quant. Biol.* **61**, 135–145.
- Rakow, N. A. & Suslick, K. S. (2000) *Nature* **406**, 710–713.
- Goodey, A., Lavigne, J. J., Savoy, S. M., Rodriguez, M. D., Currey, T., Tsao, A., Simmons, G., Wright, J., Yoo, S.-J., Sohn, Y., *et al.* (2001) *J. Am. Chem. Soc.* **123**, 2559–2570.
- Wulfschuhle, J. D., Liotta, L. A. & Petricoin, E. F. (2003) *Nat. Rev. Cancer* **3**, 267–276.
- Pweletz, C. P., Gillespie, J. W., Ornstein, D. K., Simone, N. L., Brown, M. R., Cole, K. A., Wang, Q.-H., Huang, J., Hu, N., Yip, T.-T., *et al.* (2000) *Drug. Dev. Res.* **49**, 34–42.
- Petricoin, E. F., Wulfschuhle, J., Espina, V. & Liotta, L. A. (2004) *J. Proteome Res.* **3**, 209–217.
- Barglow, K. T. & Cravatt, B. F. (2004) *Chem. Biol.* **11**, 1523–1531.