US007871777B2

(12) **United States Patent**
**Schneider et al.**

(10) **Patent No.: US 7,871,777 B2**
(45) **Date of Patent: Jan. 18, 2011**

(54) **PROBE FOR NUCLEIC ACID SEQUENCING AND METHODS OF USE**

(75) Inventors: **Thomas D. Schneider**, Frederick, MD (US); **Ilya G. Lyakhov**, Frederick, MD (US); **Danielle Needle**, Frederick, MD (US)

(73) Assignee: **The United States of America as represented by the Department of Health and Human Services**, Washington, DC (US)

( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 126 days.

(21) Appl. No.: **12/095,973**

(22) PCT Filed: **Dec. 12, 2006**

(86) PCT No.: **PCT/US2006/047534**

§ 371 (c)(1), (2), (4) Date: **Jun. 3, 2008**

(87) PCT Pub. No.: **WO2007/070572**

PCT Pub. Date: **Jun. 21, 2007**

(65) **Prior Publication Data**

US 2008/0299565 A1 Dec. 4, 2008

**Related U.S. Application Data**

(60) Provisional application No. 60/749,729, filed on Dec. 12, 2005, provisional application No. 60/749,858, filed on Dec. 12, 2005.

(51) **Int. Cl.**
***C12Q 1/68*** (2006.01)
***C07H 21/02*** (2006.01)
***C07H 21/04*** (2006.01)

(52) **U.S. Cl.** ..... **435/6**; 536/23.1; 536/24.3

(58) **Field of Classification Search** ..... 435/6; 536/23.1, 24.3
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

| | | | | |
|---|---|---|---|---|
| 5,886,177 | A | 3/1999 | Cook et al. | |
| 5,998,204 | A | 12/1999 | Tsien et al. | |
| 6,150,176 | A | 11/2000 | Tsien et al. | |
| 6,197,928 | B1 | 3/2001 | Tsien et al. | |
| 6,210,896 | B1 | 4/2001 | Chan | |
| 6,495,664 | B1 | 12/2002 | Cubitt | |
| 6,608,189 | B1 | 8/2003 | Tsien et al. | |
| 6,682,893 | B2 | 1/2004 | Taylor et al. | |
| 6,766,183 | B2 | 7/2004 | Walsh et al. | |
| 6,790,632 | B2 | 9/2004 | Zweig | |
| 6,890,556 | B1 | 5/2005 | Segura et al. | |
| 6,908,736 | B1 | 6/2005 | Densham | |
| 7,056,661 | B2 | 6/2006 | Korlach et al. | |
| 7,056,676 | B2 | 6/2006 | Korlach et al. | |
| 7,211,414 | B2 | 5/2007 | Hardin et al. | |
| 7,297,518 | B2 | 11/2007 | Quake et al. | |
| 2001/0008766 | A1 | 7/2001 | Daunert et al. | |
| 2002/0137062 | A1 * | 9/2002 | Williams et al. | 435/6 |
| 2002/0165364 | A1 | 11/2002 | Tsien et al. | |
| 2002/0193672 | A1 | 12/2002 | Walsh et al. | |
| 2003/0064366 | A1 | 4/2003 | Hardin et al. | |
| 2003/0170767 | A1 | 9/2003 | Cubitt | |
| 2003/0212265 | A1 | 11/2003 | Tsien et al. | |
| 2004/0265902 | A1 | 12/2004 | Fricker et al. | |
| 2005/0267326 | A1 | 12/2005 | Loeb et al. | |
| 2006/0112440 | A1 | 5/2006 | Tsien et al. | |
| 2006/0211075 | A1 | 9/2006 | Lawrence et al. | |

FOREIGN PATENT DOCUMENTS

| | | | |
|---|---|---|---|
| WO | WO 98/40477 | A1 | 9/1998 |
| WO | WO 00/70073 | A1 | 11/2000 |
| WO | WO 01/16375 | A2 | 3/2001 |
| WO | WO 02/04680 | A2 | 1/2002 |
| WO | WO 02/090987 | A2 | 11/2002 |
| WO | WO 2004/074503 | A2 | 9/2004 |

OTHER PUBLICATIONS

Metzker et al., Termination of DNA synthesis by novel 3'-modified deoxyribonucleoside 5'-triphosphates. Nucl. Acids Res.. 22: 4259-4267 (1994).*

(Continued)

*Primary Examiner*—Ethan Whisenant
(74) *Attorney, Agent, or Firm*—Klarquist Sparkman, LLP

(57) **ABSTRACT**

A nanoprobe for sequencing of nucleic acid molecules is provided, as well as methods for using the nanoprobe. In particular examples, the probe includes a polymerizing agent and one or more molecular linkers that carry a chemical moiety capable of reversibly binding to the template strand of a nucleic acid molecule, without being detached from the linker, by specifically binding with a complementary nucleotide in the target nucleic acid molecule. The reversible binding of the chemical moiety on the linker with a complementary nucleotide in the target nucleic acid molecule is indicated by emission of a characteristic signal that indicates pairing of the chemical moiety on the linker with its complementary nucleotide. An example of such a chemical moiety is a non-hydrolyzable nucleotide analog. In particular examples, the polymerizing agent and the chemical moiety are associated with a tag, such as a donor fluorophore and acceptor fluorophore characteristic of the particular type of chemical moiety.

**69 Claims, 12 Drawing Sheets**