lower than that of the insect tissue (*17*), the amino acids detected are likely to be endogenous. The surprising preservation of the amino acid stereochemistry in amber-entombed insects may be due to the anhydrous nature of the amber matrix. Because depurination of DNA would similarly be inhibited by anhydrous conditions, an amber matrix may provide conditions conducive to the long-term preservation of nucleic acids.

REFERENCES AND NOTES

1. K. B. Mullis and F. Faloona, *Methods Enzymol.* **155**, 335 (1987).
2. S. Pääbo, R. G. Higuchi, A. C. Wilson, *J. Biol. Chem.* **264**, 9709 (1989).
3. S. Pääbo, *Sci. Am.* **269**, 86 (November 1993).
4. H. N. Poinar, R. J. Cano, G. O. Poinar Jr., *Nature* **363**, 677 (1993); R. J. Cano, H. N. Poinar, N. J. Pieniazek, A. Acra, G. O. Poinar Jr., *ibid*, p. 536; R. DeSalle, J. Gatesy, W. Wheeler, D. Grimaldi, *Science* **257**, 1933 (1992); S. R. Woodward, N. J. Weyand, M. Bunnell, *ibid.* **266**, 1299 (1994); S. Richardson, *Discover* (January), 55 (1994); E. M. Golenberg *et al.*, *Nature* **344**, 656 (1990); P. S. Soltis, D. E. Soltis, D. F. Smiley, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 449 (1992).
5. M. Höss, A. Dilling, A. Currant, S. Pääbo, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 181 (1996).
6. O. Handt, M. Höss, M. Krings, S. Pääbo, *Experientia* **50**, 524 (1994).
7. S. Pääbo and A. C. Wilson, *Curr. Biol.* **1**, 45 (1991); T. Lindahl, *Nature* **362**, 709 (1993); A. Sidow, A. C. Wilson, S. Pääbo, *Philos. Trans. R. Soc. London Ser. B* **333**, 429 (1991); H. Zischler *et al.*, *Science* **268**, 1192 (1995); R. V. Collura and C. B. Stewart, *Nature* **378**, 485 (1995).
8. T. Lindahl, *Nature* **365**, 700 (1993); M. Höss, M. K. Vereshchagin, S. Pääbo, *ibid.* **370**, 333 (1994).
9. J. L. Bada, *J. Am. Chem. Soc.* **94**, 1371 (1972); ———, K. A. Kvenvolden, E. Peterson, *Nature* **245**, 208 (1973); J. L. Bada and E. H. Man, *Earth Sci. Rev.* **16**, 21 (1980); J. Bada, in *Chemistry and Biochemistry of Amino Acids*, G. C. Barrett, Ed. (Chapman and Hall, London, 1985), pp. 399–414; *Annu. Rev. Earth Sci.* **13**, 241 (1985); *Philos. Trans. R. Soc. London Ser. B* **333**, 349 (1991).
10. T. Lindahl and B. Nyberg, *Biochemistry* **11**, 3610 (1972); T. Lindahl and A. Anderson, *ibid.*, p. 3618.
11. J. Bada, X. S. Wang, H. N. Poinar, S. Pääbo, G. O. Poinar Jr., *Geochim. Cosmochim. Acta* **58**, 3131 (1994).
12. External surfaces of bone sections (~1 mm) were removed, and samples were ground under liquid nitrogen in a Freezer/Mill 6700 bone grinder (Spex Industries, Edison, NJ). Then 0.01 to 0.5 g were hydrolyzed in doubly distilled 6 N HCl for 24 hours at 100°C. Glassware was cleaned by immersion in 10 M HCl for 2 weeks, then rinsed in doubly distilled water and baked at 250°C for 1 week. Soft tissue samples were briefly rinsed in 0.01 N HCl and hydrolyzed as above. Subsequently, samples were dried under vacuum over NaOH. Bone samples were redissolved in doubly distilled water and desalted with a cation exchanger (50W-X8) (Bio-Rad) as in J. L. Bada, *Earth Planet. Sci. Lett.* **15**, 223 (1972). Soft tissue samples were dissolved directly in 0.4 M sodium borate. Amino acids were derivatized with *O*-phthaldialdehyde/*N*-acetyl-L-cysteine (OPA/NAC) and analyzed by high-pressure liquid chromatography (HPLC) (Gilson, Middleton, WI) with fluorescent detection as described in M. Zaho and J. L. Bada, *J. Chromatogr. A* **690**, 55 (1995). Mock samples were analyzed in parallel with each series of samples, and background amino acid concentrations were subtracted from sample values. For quantitation and accurate determination of enantiomeric ratios, a standard containing a racemic mixture of the selected amino acids was analyzed on the same day as the samples. To determine the extent of racemization caused by the experimental procedure, we analyzed five bovine serum albumin (Pharmacia) samples; they were found to have a D/L ratio for Asp of 0.034 ± 0.0035. Variation due to sample processing was tested by the analysis of 10 samples from the same bone, yielding a D/L ratio of 0.056 ± 0.0036. We investigated the relative fluorescence of OPA-derivatized D and L forms of Asp, Ala, and Leu by analyzing samples with D/L ratios of 1. The observed ratios (0.867, 0.85, and 0.80, respectively) were used to adjust values determined for the samples.
13. M. Höss, P. Jaruga, T. H. Zastawny, M. Dizdaroglu, S. Pääbo, *Nucleic Acids Res.* **24**, 1304 (1996).
14. For example, the total amounts of the six amino acids analyzed [Asp, serine (Ser), Ala, Gly, Leu, and valine (Val)] do not correlate with the retrieval of DNA sequences (H. N. Poinar, M. Höss, J. L. Bada, S. Pääbo, data not shown). Furthermore, a ratio of Gly to Asp of 5.5 or larger can be used as a rough estimate of the preservation of collagen in bone [H. Elster, E. Gil-Av, S. Weiner, *J. Archeol. Sci.* **18**, 605 (1991)]. However, DNA could be extracted both from bones in which collagen was preserved according to this criterion and from some in which it was not.
15. Only minute amounts of amino acids were present in these samples, and these values were not significantly different from those in the surrounding sediment. As a result, the D/L ratios for Asp could not be determined in most cases. The presence of bacterial amino acid decomposition products, for example, β-alanine and γ-amino-*n*-butyric acid, in these specimens also suggests bacterial contamination (J. L. Bada, unpublished observations).
16. G. A. Logan, J. J. Boon, G. Eglinton, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2246 (1993); G. A. Logan and G. Eglinton, *Org. Geochem.* **21**, 857 (1994).
17. X. S. Wang, H. N. Poinar, G. O. Poinar Jr., J. L. Bada, in *Amber, Resinite, and Fossil Resin*, K. B. Anderson and J. C. Crelling, Eds. (American Chemical Society, Washington, DC, 1995), pp. 255–262.
18. M. Höss and S. Pääbo, *Nucleic Acids Res.* **21**, 3913 (1993).
19. A. Cooper and P. Hood, in *Avian Molecular Evolution and Systematics*, D. Mindell, Ed. (Academic Press, New York, in press).
20. The Utah and Montana dinosaur samples were too small to allow for the removal of surface material. Instead, they were rinsed several times with 0.01 N HCl and then with doubly distilled water before hydrolysis. The remaining dinosaur bones were processed like the other bones (*12*). Clarkia sediments containing fossil leaves were opened in a hood, photographed, and rinsed with 0.01 N HCl. Leaves were then scraped off into sterile vials, hydrolyzed, desalted, and analyzed by HPLC (*12*). Surrounding sediments were similarly analyzed. Amber samples were processed as in (*11*).

21. We thank V. Börner, A. Cooper, O. Handt, M. Krings, G. O. Poinar, J. Rea, A. Sajantila, S. Weiner, S. Woodward, H. Zischler, and others for help, and J. E. Cadle, A. Currant, A. v.d. Driesch, U. Joger, H. G. McDonald, M. C. McKenna, C. E. Ray, C. A. Shaw, A. J. Sutcliffe, H. P. Uerpmann, M. K. Vereschagin, and M. Hanczyk for samples. This research was supported by the Deutsche Forschungsgemeinschaft (S.P.) and the NASA Specialized Center for Research and Training in Exobiology (J.L.B.).

18 December 1995; accepted 12 March 1996

Polymers with Very Low Polydispersities from Atom Transfer Radical Polymerization

Timothy E. Patten, Jianhui Xia, Teresa Abernathy, Krzysztof Matyjaszewski*

A radical polymerization process that yields well-defined polymers normally obtained only through anionic polymerizations is reported. Atom transfer radical polymerizations of styrene were conducted with several solubilizing ligands for the copper(I) halides: 4,4′-di-*tert*-butyl, 4,4′-di-*n*-heptyl, and 4,4′-di-(5-nonyl)-2,2′-dipyridyl. The resulting polymerizations have all of the characteristics of a living polymerization and displayed linear semilogarithmic kinetic plots, a linear correlation between the number-average molecular weight and the monomer conversion, and low polydispersities (ratio of the weight-average to number-average molecular weights of 1.04 to 1.05). Similar results were obtained for the polymerization of acrylates.

Szwarc and Levy first reported on living anionic polymerizations in 1956 (*1*, *2*), and this technique made it possible to prepare well-defined polymers. A living polymerization is a chain-growth polymerization that propagates with no irreversible chain-transfer or chain-termination reactions. Provided that initiation is fast, the degree of polymerization is proportional to the ratio of the concentrations of monomer consumed and initiator, and the product polymer has a polydispersity that approaches a Poisson distribution. The discovery of living polymerizations revolutionized synthetic polymer chemistry, because with such polymerizations one can prepare block and triblock copolymers, end-functionalized polymers, and star polymers (*3*). Of the known living ionic and metal-catalyzed polymerizations, living anionic polymerization affords the best control over the resulting polymers and provides polymers with the narrowest molecular weight distribution (ratio of the weight-average to number-average molecular weight, M_w/M_n).

Radical polymerizations are important for the industrial production of commodity polymers, which account for a major fraction of polymer industry's annual production, yet are very difficult to control because of fast, irreversible termination of the growing radicals through coupling and disproportionation reactions. Because of these

Department of Chemistry, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213, USA.

*To whom correspondence should be addressed.

termination reactions, radical polymerizations have eluded attempts to convert them into living polymerizations.

Recently, controlled radical polymerizations have been reported in which this fast termination problem has been circumvented by one of several methods. In general, there are three categories of controlled radical polymerization (*4*). The first type is based on the reversible homolytic cleavage of dormant chain ends to form a radical chain end and a stable radical that cannot initiate polymerization. Examples of such polymerizations include the TEMPO-mediated (TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical) polymerization of styrene (*5*, *6*) and the polymerization of acrylates catalyzed by Co porphyrin alkyls (*7*, *8*). The second class is based on reversible complexation of the active chain ends to form persistent radicals; this process has been proposed in the polymerization of vinyl acetate mediated by an alkyl Al–TEMPO complex (*9*). The third type is based on bimolecular degenerative transfer of an end group between active and dormant chains, as demonstrated recently in the I-atom–mediated polymerization of styrene (*10*). In each of these examples, one "mediating group" (metal or nitroxyl) is needed to control the reactivity of one chain end, which can be quite expensive. Furthermore, each of the aforementioned techniques tends to be specific to a particular type of monomer.

Recently Wang and Matyjaszewski reported on a controlled radical polymerization technique, atom transfer radical polymerization (ATRP) (*11*, *12*), in which a catalytic amount of a Cu(I) coordination complex reversibly abstracts an X atom (X = Cl, Br) from the polymer chain ends, switching them from a dormant state to an active, propagating state. A similar atom transfer method that uses a two-component catalyst system $[RuCl_2(PPh_3)_3$–$MeAl(ODBP)_2$, where Ph is phenyl, Me is methyl, and ODBP is 2, 6-di-*tert*-butyl phenoxide] has been used successfully in the polymerization of methyl methacrylate (*13*). The ATRP process exhibits all of the experimental criteria of a living polymerization and is general for a large number of monomers. Thus far, ATRP can be used to prepare both oligomers and polymers with molecular weights up to $M_n = 10^5$ and with polydispersities ranging from 1.15 to 1.5. We now report that ATRP can be modified such that one can prepare polymers with polydispersities equal to those found for the most carefully performed living anionic polymerizations (1.05 or less).

The principle of ATRP, and of all controlled radical polymerizations, is to establish a dynamic equilibrium between active and dormant chain ends (Eq. 1)

$$P_n\text{–}X \underset{k_{deact}}{\overset{k_{act}}{\rightleftharpoons}} P_n\cdot + X\cdot \quad (1)$$

$P_n\cdot + \text{Monomer} \xrightarrow{k_p}$

where P_n is a polymer chain of n units. Thermodynamically, the equilibrium must lie toward the side of dormant chain ends to maintain a sufficiently low steady-state concentration of radicals such that bimolecular termination is minimized. Kinetically, the exchange between dormant and active polymer chain ends must be fast, otherwise not all of the chains will grow at the same rate and the polydispersity will increase. In the case of ATRP, the initiator and dormant polymer chains have a halide end group, and activation of these chains is achieved through a catalytic process. In the proposed catalytic cycle (Scheme 1), a Cu(I) halide–2,2′-dipyridyl (bipy) complex reversibly abstracts halogen atoms from the dormant chain ends, which generates a small concentration of polymer-based radicals and the corresponding Cu(II) halide complex. These radicals can then add monomer until they abstract a halogen atom from the Cu(II) complex, thus completing the dormant-to-active chain-end catalytic cycle. Termination in any radical polymerization is unavoidable; however, the activation-deactivation cycle in ATRP minimizes termination by creating a steady, low concentration of short-lived, active radical chain ends.

In a typical ATRP of styrene, either 1-phenylethyl chloride or bromide (1 mol % based on styrene for [monomer]/[initiator] = 100, brackets denote concentration) is used as the initiator and ~1 mol % CuX (X = Cl, Br) with two equivalents of bipy is used as the catalyst. Upon heating (130°C for X = Cl, 110°C for X = Br) in a sealed tube for 5 hours, the bulk polymerization proceeds to ~95% conversion (*11*). The M_n evolves linearly with conversion and linearly as a function of the initial monomer-to-initiator ratio, and the polydispersities of the resulting polymer range from 1.15 to 1.5. The Cu–bipy complex in these polymerizations is only slightly soluble in the reaction medium, and thus ATRP with this particular catalyst system is a heterogeneous process (*12*).

We sought to increase the solubility of the Cu(I) halide in the polymerization medium by adding solubilizing side chains to the 4,4′-positions of the bipy ligand. Alkyl chains of at least four C centers are satisfactory for this purpose. When ligands such as 4,4′-di-*tert*-butyl (dTbipy) (*14*), 4,4′-di-*n*-heptyl (dHbipy) (*15*), and 4,4′-di-(5-nonyl)-2,2′-dipyridyl (dNbipy) were used in place of bipy for ATRP, we observed a marked lowering of the polydispersities of the resulting polymers. For example, in a polymerization similar to the one described above except that dHbipy was used instead of bipy, the polydispersity of the polymer was 1.04. By comparison, commercially available, anionically prepared polystyrene standards for use in the calibration of gel permeation chromatography (GPC) columns have polydispersities in the range of 1.03 to 1.05 (Fig. 1).

When we monitored this polymerization using gas chromatography (GC) and GPC, the polymerization kinetics were first-order with respect to styrene for more than four half-lives. There was also a linear correspondence between the molecular weight of the polymer and the monomer conversion. The experimentally determined molecular weights correlated well with the expected molecular weights, and the polydispersities consistently remained around 1.05 (±0.01). Figure 2 shows how the GPC chromatograms evolve during the course of homogeneous ATRP. For

Initiation:

$$R\text{–}X + \text{“2 bipy/CuX”} \rightleftharpoons R\cdot + \text{“2 bipy/CuX}_2\text{”} \quad (X = Cl, Br)$$

$$R\cdot + \text{monomer} \longrightarrow P_1\cdot$$

Propagation:

$$P_n\text{–}X + \text{“2 bipy/CuX”} \rightleftharpoons P_n\cdot + \text{“2 bipy/CuX}_2\text{”}$$

$$P_n\cdot + \text{monomer} \longrightarrow P_{n+1}\cdot$$

Termination:

$$P_n\cdot + P_m\cdot \longrightarrow P_{n+m} + (P_n^{2-} + P_mH)$$

Scheme 1.

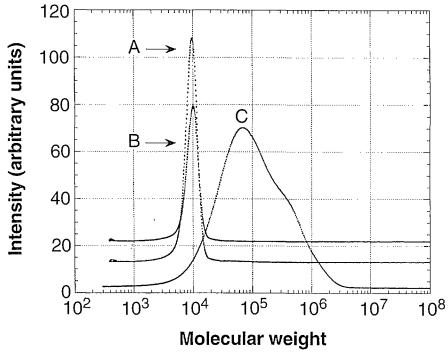

Fig. 1. Comparison of GPC chromatograms for a series of polystyrenes: curve A, GPC standard (M_w/M_n = 1.05); curve B, bulk homogeneous ATRP (M_w/M_n = 1.04) for conditions described in Fig. 2; curve C, bulk free-radical polymerization initiated by $[AIBN]_0$ = 0.13 M at 60°C (M_w/M_n = 1.84) (AIBN, azobisisobutyronitrile).

these molecular weight studies, the polymerization solutions were diluted with tetrahydrofuran and injected directly into the GPC in order to avoid any fractionation of the polymer samples during isolation, which would artificially narrow the molecular weight distributions. This system can also be used for the synthesis of well-defined polyacrylates. For example, the bulk polymerization of methyl acrylate (initial concentrations [methyl α-bromopropionate]$_0$ = 0.03 M, [CuBr]$_0$ = 0.03 M, and [dHbipy]$_0$ = 0.06 M) after 1.5 hours at 110°C yields a polymer with M_n = 17,000 and M_w/M_n = 1.05.

Generally, homogeneous ATRP is useful for synthesizing various polymers up to a molecular weight of 50,000 to 80,000. Above this molecular weight range some limiting side reactions can be noticed. We have also used this system to polymerize other vinyl monomers; to prepare block, random, graft, and gradient copolymers; and to synthesize macromolecules with various topologies (branched, hyperbranched, and star polymers) (*16*).

Computer simulations of the experimental kinetic and molecular weight data indicate that <3% of the chains are terminated at >99% monomer conversions. In addition, because the polymerizations are fast, the proportion of chains formed by thermal self-initiation of styrene does not exceed 1%. Thus, nearly all of the chains are either in the dormant or active state throughout the polymerization, fulfilling the requirements for a living polymerization. However, because termination undoubtedly occurs, we prefer to use the term "controlled" or living polymerization.

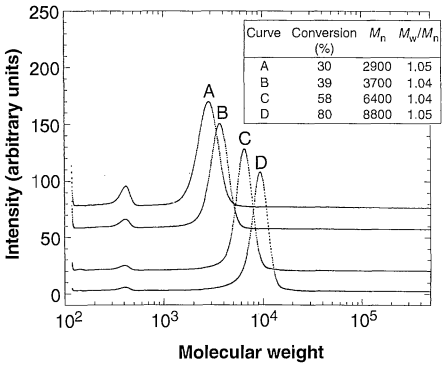

Fig. 2. Evolution of the GPC chromatograms (curves A through D) as a function of conversion for an ATRP of bulk styrene at 110°C using [CuBr]$_0$ = 8.4 × 10^{-2} M, [1-PEBr]$_0$ = 8.8 × 10^{-2} M ([monomer]$_0$/[initiator]$_0$ = 100), and [dHbipy]$_0$ = 17.5 × 10^{-2} M (1-PEBr = 1-phenylethyl bromide). An 80% conversion (GPC chromatogram D) was reached after 4 hours. Similar behavior has been observed for ligands dNbipy and dTbipy. The small signals in the low molecular weight range in each chromatogram are due to the residual catalyst used in these polymerizations (a result of injecting the diluted polymerization mixture directly into the GPC apparatus).

These simulations also show that the polydispersities are strongly affected by the ratio of the rates of deactivation to propagation. In these studies, the new ligands increase the solubility of both the Cu(I) and the Cu(II) species in the polymerization medium [commercial CuBr contains ~1 to 2% Cu(II)]. The latter species acts as a deactivator, and therefore its presence initially in the polymerization maintains a sufficiently high rate of deactivation relative to propagation throughout the polymerization. As a consequence, the experimentally observed polydispersities remain low. When polymerizations are conducted with very pure Cu(I) halide, containing no Cu(II) halide, the polydispersities of the resulting polymers increase (>1.10).

REFERENCES AND NOTES

1. M. Szwarc and M. Levy, *J. Am. Chem. Soc.* **78**, 2657 (1956).
2. M. Szwarc, *Nature* **178**, 1168 (1956).
3. O. W. Webster, *Science* **251**, 887 (1991).
4. D. Greszta, D. Mardare, K. Matyjaszewski, *Macromolecules* **27**, 638 (1994).
5. E. Rizzardo, *Chem. Aust.* **1987**, 32 (1987).
6. M. K. Georges, R. P. N. Veregin, P. M. Kazmaier, G. K. Hamer, M. Saban, *Macromolecules* **27**, 7228 (1994).
7. H. J. Harwood, L. D. Arvanitopoulos, M. P. Greuel, *Polym. Prepr. Am. Chem. Soc. Div. Polym. Chem.* **35**, 549 (1994).
8. B. B. Wayland, G. Pszmik, S. L. Mukerjee, M. Fryd, *J. Am. Chem. Soc.* **116**, 7943 (1994).
9. D. Mardare and K. Matyjaszewski, *Macromolecules* **27**, 645 (1994).
10. S. Gaynor, J. S. Wang, K. Matyjaszewski, *ibid.* **28**, 8051 (1995).
11. J. S. Wang and K. Matyjaszewski, *J. Am. Chem. Soc.* **117**, 5614 (1995).
12. ———, *Macromolecules* **28**, 7901 (1995).
13. M. Kato, M. Kamigaito, M. Sawamoto, T. Higashimura, *ibid.*, p. 1721.
14. T. B. Hadda and H. L. Bozec, *Polyhedron* **7**, 575 (1988).
15. Synthesized according to the procedure in R. Kramer, J. M. Lehn, A. D. Cian, J. Fisher, *Angew. Chem. Intl. Ed. Engl.* **32**, 703 (1993).
16. K. Matyjaszewski *et al.*, U.S. patent pending.

17. This work was funded in part by the Office of Naval Research.

21 December 1995; accepted 6 March 1996

Homologous DNA Pairing Promoted by a 20–Amino Acid Peptide Derived from RecA

Oleg N. Voloshin, Lijiang Wang, R. Daniel Camerini-Otero*

The molecular structure of the *Escherichia coli* RecA protein in the absence of DNA revealed two disordered or mobile loops that were proposed to be DNA binding sites. A short peptide spanning one of these loops was shown to carry out the key reaction mediated by the whole RecA protein: pairing (targeting) of a single-stranded DNA to its homologous site on a duplex DNA. In the course of the reaction the peptide bound to both substrate DNAs, unstacked the single-stranded DNA, and assumed a β structure. These events probably recapitulate the underlying molecular pathway or mechanism used by homologous recombination proteins.

Homologous recombination is the central phenomenon underlying genetic processes and the principal tool in genetic analysis, gene mapping, and gene targeting. The key reaction in homologous recombination is the pairing of any two arbitrary but homologous DNA molecules. Prokaryotic RecA protein plays an essential role in bacterial homologous recombination pathways and is the prototype for homologous DNA pairing proteins (*1, 2*). RecA is a 38-kD polypeptide with diverse biochemical activities, including the promotion of DNA pairing in a reaction that involves three strands (*3*). RecA binds to single-stranded DNA (ssDNA), and the resulting nucleoprotein (presynaptic) filament is the homology-searching moiety that mediates the pairing with a target duplex. The DNAs that become paired reside within a hole 25 Å in diameter as seen in projection down the axis of a protein helix consisting of six protomers per turn (*4*). Lining this cavity and projecting toward the helix axis are two disordered or mobile loops, L1 and L2, that have been proposed as DNA binding sites (*5*).

Several lines of evidence indicate that loop L2 is a DNA binding domain. (i) Proteolysis of ssDNA-RecA complexes yields a unique 4-kD peptide protected by the DNA that spans this loop (*6*); (ii) photocrosslinks between a 5-iododeoxyuridine–containing ssDNA and RecA map to loops L1 and L2 (*7*); (iii) the fluorescence of a tryptophan substituted for the central phenylalanine in loop L2 is quenched in RecA-DNA complexes (*8*); and (iv) the 20–amino acid FECO peptide (Fig. 1) binds

Genetics and Biochemistry Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892–1810, USA.

*To whom correspondence should be addressed.