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## ORIGINAL ARTICLE

# Synthetic polymers as substrates for a DNA-sliding clamp protein

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## Abstract

The clamp protein (gp45) of the DNA polymerase III of the bacteriophage T4 is known to bind to DNA and stay attached to it in order to facilitate the process of DNA copying by the polymerase. As part of a project aimed at developing new biomimetic data-encoding systems we have investigated the binding of gp45 to synthetic polymers, that is, rigid, helical polyisocyanopeptides. Molecular modelling studies suggest that the clamp protein may interact with the latter polymers. Experiments aimed at verifying these interactions are presented and discussed.

## 1 | INTRODUCTION

Nature employs highly specialized processive catalysts to create or modify biopolymers in a selective and precise fashion. Processive catalysts are catalysts that bind to a template and perform multiple rounds of catalysis before they dissociate.<sup>[1]</sup> The efficiency of nature's processive catalysts is stunning, and even small lessons taken from nature could resonate astoundingly when applied to polymer chemistry: the demand for new catalyst technology is perennial, yet only few artificial processive catalysts exist.<sup>[2–9]</sup>

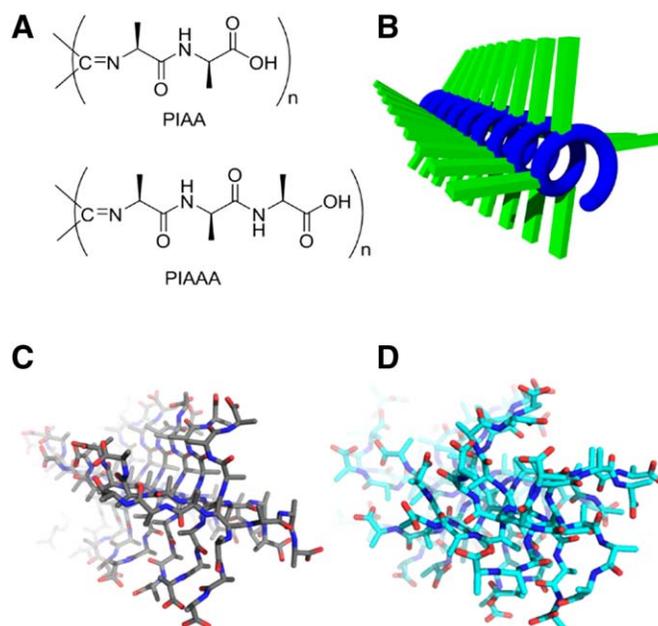
Different types of processive catalysts play crucial roles during the life cycle of a cell, such as DNA or RNA polymerases and helicases.<sup>[10,11]</sup> Central to many of these metabolic processes are clamp-shaped proteins that can encircle DNA to initiate a mechanical bond, and then recruit other proteins or enzymes through specific binding

interactions.<sup>[12]</sup> As such, protein clamps can confer processive behavior onto otherwise distributive catalysts. We have recently reported a strategy in which the trimeric ring-shaped clamp of the bacteriophage T4 (gp45)<sup>[13]</sup> is used to impart processivity to an otherwise distributive porphyrin catalyst.<sup>[7]</sup> The combination of this porphyrin with the DNA-tracking behavior of the T4 clamp protein produced a biohybrid catalyst for the processive modification of DNA. In an effort to extend this platform, we describe here studies aimed at enabling the clamp protein to accept not only nucleic acids but also synthetic polymers as substrates. Such behavior is an important next step in the development of artificial processive catalysts that can encode biopolymers and synthetic polymers with information.

The T4 clamp will not randomly associate with any polymer it encounters. The cell itself is filled with all sorts of polymers and these do not seem to distract the clamp. In fact, the clamp does not even

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**FIGURE 1** A, Structural formulae of polyisocyanopeptides PIAA and PIAAA. B, Cartoon representation detailing their helical backbone (blue) and their side chain  $\beta$ -sheets (green). C, Organization of PIAAA before and D, after molecular dynamics modelling

associate with all nucleic acids at all times: a dedicated clamp-loader protein complex is needed for the loading process. This complex (gp44/62) recruits the clamp, opens it, slides it onto the DNA chain, after which the clamp closes and the loader complex dissociates.<sup>[14,15]</sup> This process is driven by ATP hydrolysis, and *in vitro* assays quantifying hydrolysis have often been used to determine the frequency of the loading events.<sup>[7,14–16]</sup> It stands to reason that a synthetic polymer's suitability to act as a substrate depends not only on its interaction with the gp45 clamp, but also on its interaction with the clamp loader, which opens the clamp, and its ability to stimulate ATP hydrolysis as an experimental measure of the clamp loading process onto a synthetic polymer.

What type of polymers might replace DNA in its interaction with the clamp protein? No studies of clamp proteins with synthetic polymers have been reported in the literature. To direct our thinking, we may look at the way synthetic polymers interact with virus proteins and how they can act as templates for virus assembly. For example, it has been reported that viral capsid proteins can assemble around polystyrene sulfonate<sup>[17,18]</sup> and anionic polyferrocenylsilane.<sup>[19]</sup> The lessons from these studies seem to be that the polyanionic character of the artificial polymer is of paramount importance for achieving interactions with the protein molecules. In light of this, a class of candidate polymers that shares many of its characteristics with polynucleotides is that of the polyisocyanopeptides. These polymers are prepared from isocyanopeptides by the catalytic action of nickel salts. They may have either two or three alanine residues in their side chains (abbreviated as PIAA and PIAAA, respectively, Figure 1A).<sup>[20–23]</sup> These unique polymers are helical and anionic at physiological pH. Furthermore, they have an apparent diameter and charge spacing that compare well to the values for dsDNA. Compared to most synthetic polymers, the persistence length of PIAA(A) is also closer to that of dsDNA (see Supporting

Information Table S1). Moreover, the helical morphology of PIAA(A), which is strengthened by the miniature  $\beta$ -sheets formed by its side chains, makes it resemble dsDNA even more. In this article, we describe molecular modelling studies, which suggest that polyisocyanopeptides may interact with DNA accessory proteins, that is, the gp45 clamp. Our experimental studies reveal, however, that these interactions are present, but are relatively weak.

## 2 | EXPERIMENTAL

### 2.1 | Materials and methods

#### 2.1.1 | Materials

DNA substrates were obtained from IDT (Coralville, IA). Steptavidin was purchased from Sigma (St. Louis MO). Restriction enzymes were obtained from New England Biolabs (Ipswich, MA). All other chemicals were analytical grade or better. PIAA and PIAAA were a kind gift from Dr. G. Metselaars, and were synthesized as described in the literature.<sup>[22–24]</sup> Solutions of these polymers were prepared as follows. The respective methoxy-protected polyisocyanide (12 mg) was suspended in an aqueous solution of 0.5M NaOH (5 mL). The suspension was stirred for 4 h, until an opaque solution was obtained. A small amount of this solution was mixed with the buffer and used in the binding experiments, see below.

#### 2.2 | Structure preparation/dataset for simulations

The polyisocyanopeptide structures were drawn in the molecular modeling software package Spartan.<sup>[25]</sup> Each PIAA and PIAAA monomer was assigned to a residue number. Both structures contained 64 residues forming a  $4_1$  helix with side chains  $n$  and  $n + 4$  stacked on top of each other. Each residue has a carboxyl end group, which is

deprotonated at physiological conditions. In the polyisocyanopeptides the  $pK_a$  value of the carboxylates is influenced by the surrounding carboxylates, therefore not all carboxylates can be deprotonated. Keeping this in mind the  $pK_a$  of residues  $n$  to  $n + 3$ , in each alternate tetramer was set to 9. This resulted in protonated carboxylates. The clamp-clamp loader complex used was from Bacteriophage T4. The coordinates of DNA polymerase accessory protein 44 (chains B, C, and D (without ADP) and E), DNA polymerase accessory protein 62 (chain A) and the DNA polymerase processivity component (chains F, G, H) were copied from the PDB structure 3U5Z. The DNA inside the clamp/clamp loader complex was replaced with the LD-PIAA and LDL-PIAAA structures, preparing two separate complexes.

### 2.3 | Force field and molecular dynamics simulations protocol

Molecular dynamics simulations were performed in the YASARA structure software package, version 14.2.3.<sup>[26]</sup> YASARA utilizes an automatic parameterization algorithm, AutoSMILES, to generate force field parameters for organic molecules. The algorithm is described in detail on the YASARA website.<sup>[27]</sup> Because the polyisocyanopeptides contained the same atom types as proteins, the force field used was AMBER03, one of the best available for protein simulations.<sup>[28]</sup> New parameters, which were not part of the AMBER03 force field, were derived using the AutoSMILES algorithm. Simulations were performed for PIAA, PIAA inside the clamp/clamp loader complex, and for PIAAA and PIAAA inside the clamp/clamp loader complex. In all cases, the simulation box was filled with TIP3P water molecules and  $Na^+$  and  $Cl^-$  ions were added to neutralize the system. The size of simulation boxes and the number of atoms in each simulation are shown in Supporting Information Table S2. To remove bumps and correct covalent geometries, all structures were initially energy-minimized without electrostatic interactions by a short steepest descent minimization. After removal of conformational stress, simulated annealing was performed (time step 2 fs, atom velocities scaled down by 0.9 every 10th step) until convergence was reached, that is, until the energy was improved by less than 0.05 kJ/mol per atom during 200 steps. The simulations were then run for 30 ns and snapshots were collected at every 25 ps. All simulations were performed at a pH of 7.4 with constant pressure and a temperature of 298 K. The boundary conditions used were periodic and the long range electrostatic forces were calculated using the Particle Mesh Ewald method.<sup>[29]</sup> The cut-off distance used for Van der Waals interactions was 7.86 Å. The water density was kept at 0.997 g L<sup>-1</sup>.

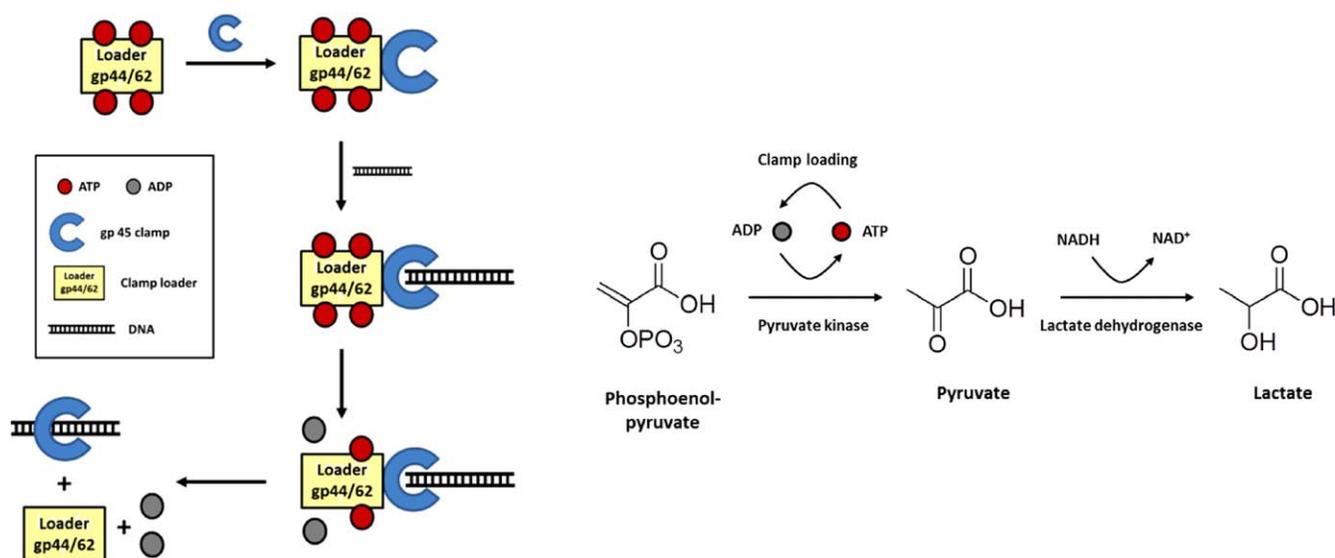
### 2.4 | Analysis of MD simulations

Distances were measured diagonally between O atoms of the terminal carboxyl groups, between pairs of residues at five different points along the polymer (Supporting Information Table S3). Note that each negatively charged PIAAA residue contains 3 alanines (PIAA contains 2 alanines) in the side chain. Each polyisocyanopeptide helical wind is composed of four such residues and the polymer as simulated contains 16 winds (64 residues) in total. The distances were measured between

the residue pairs (7,9), (17,19), (29,31), (41,43), and (53,55) (for PIAA the distances between C-alpha atoms for the residue pairs (7,9), (17,19), (29,31), (41,43), and (53,55) were also measured) to cover the polymer's length with a good distribution. Because the carboxyl groups at the ends kept wobbling, the distances of the C-alpha atoms of the first, second and third alanine were also measured between each pair of the aforementioned residues (Supporting Information Table S3). The movement of these C-alpha atoms was expected to be restricted due to the presence of H-bonds between the N-H and C=O groups. To compare the diameter of DNA with that of polyisocyanopeptides, the coordinates of double helical DNA were copied from the PDB structure 3U5Z (chain I and J). Distances were measured between the O1P atoms of the two strands S1 and S2 at five different points (Supporting Information Table S4). S1 and S2 contain 24 and 20 nucleotides respectively (the extra 4 residues in S1 form the template overhang). The average end to end distance between the O1P atoms of DNA is comparable to the average of the maximum distance between the O atoms of the end carboxyl groups of PIAAA (see Supporting Information Tables S3 and S4).

### 2.5 | ATPase experiments

ATPase experiments were performed at 23°C, essentially as described in the Supporting Information section of our previous paper (for a schematic representation of the measuring events see Figure 2).<sup>[7]</sup> Bacteriophage T4 proteins: gp45 (250 nM) and gp44/62 (250 nM) as well as the forked DNA substrate (Bio34/26/36) were prepared as previously described.<sup>[13,16]</sup> Reaction buffer consisted of 25 mM Tris acetate (pH 7.5), 150 mM potassium acetate, 10 mM magnesium acetate, 2 mM ATP, 4 mM phospho-(enol)-pyruvate (PEP), 0.2 mM NADH, and 3 U lactate dehydrogenase (LDH)/pyruvate kinase (PK) (Sigma). Fresh supercoiled pUC18 plasmid was prepared using GeneJET Midiprep kit (ThermoScientific). Nicked pUC18 was prepared with Np.BspQI (New England Biolabs, Ipswich, MA); blunt end pUC18 was prepared with SacI; and 5' overhang pUC18 was prepared with BamHI. All digested plasmids were purified using a PCR purification kit (Thermo Scientific). A typical assay was performed in a ~300 µL total volume as follows: 250 µL of a cocktail solution (buffer with ATP, phospho-(enol)-pyruvate, NADH, LDH/PK) was supplemented with 23.9 µL MilliQ and the background reaction (absorption at 340 nm) was measured. Then 1.7 µL gp44/62 solution was added and incubated for 3 min, while the absorption of NADH was monitored at 340 nm. Thereafter, 1.5 µL gp45 solution was added and the solution was incubated for at least 3 min. before 1.9 µL of (forked) DNA was added. Experiments with the polymers were performed in a similar way using 1 µL of a polymer solution (20 µg µL<sup>-1</sup>) instead of the DNA substrate. ATPase rates are reported as nM s<sup>-1</sup> and are the result of multiple independent experiments (at least 3) with included standard error. They were calculated by fitting the initial linear parts of the measured decrease of the absorbance of NADH at 340 nm as a function of time by using the least square method. Rates were converted to nM s<sup>-1</sup> by multiplying the rate in Abs/min with 2679.5 (=10<sup>9</sup>/60/ε<sub>NADH</sub>), in which ε<sub>NADH</sub> = 6220 M<sup>-1</sup> cm<sup>-1</sup>. The percent change is calculated from the



**FIGURE 2** Schematic representation of the assay used for measuring the interaction of the clamp loader protein complex with DNA or a synthetic polymer. Schematic representation of the events that take place when the clamp and clamp loader interact with the (bio)polymeric substrate (left) and spectroscopic assay for the quantitative analysis of the clamp loading process in which the consumption of ATP is measured by following the increase and decrease of NADH and NAD<sup>+</sup> (right)

percent difference of the gp44/62/45 rate in the absence of DNA or polymer. A standard student's *T* test was used to determine significant differences of ATPase rates. *P* values are reported for each experimental condition.

### 3 | RESULTS AND DISCUSSION

#### 3.1 | Molecular modelling studies

As a first step in assessing PIAA(A) as templates for the gp45 clamp protein, we decided to pursue a molecular modelling approach, also using available X-ray structures of gp45 and gp44/62 in a complex with an oligonucleotide (PDB: 3U5Z).

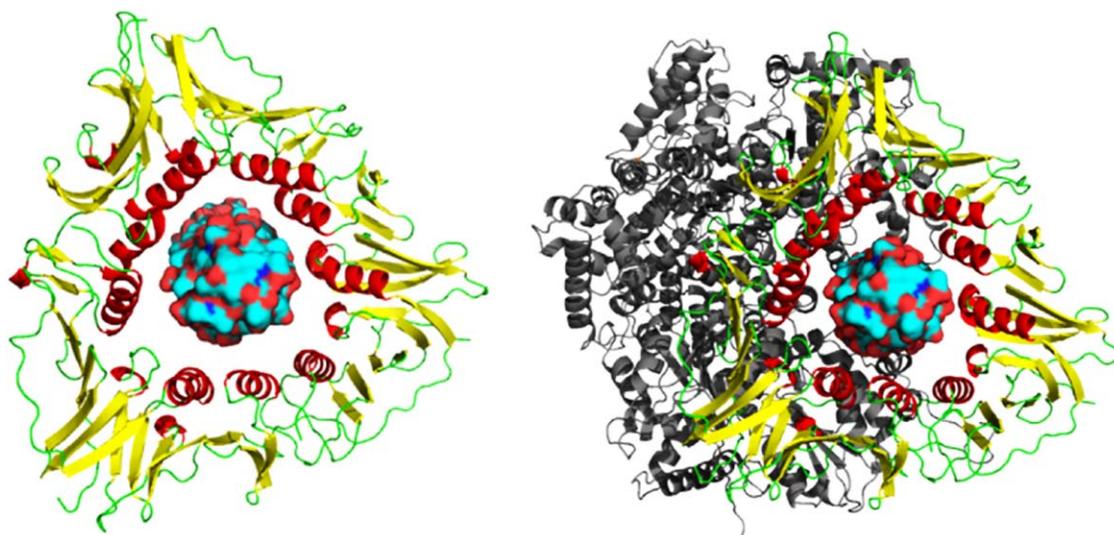
Models for PIAA(A) generated in Spartan<sup>[25]</sup> were loaded in YASARA<sup>[26]</sup> as mol2 files. Each monomer was assigned a residue number. Both structures contained 64 residues forming a 4<sub>1</sub> helix with side chains *n* and *n* + 4 stacked on top of each other (Figure 1B,C). Each residue terminated in a carboxylate group, deprotonated at physiological conditions. In PIAA(A), the p*K*<sub>a</sub> value of individual carboxylates is influenced by the surrounding ones, therefore not all carboxylates can be deprotonated. With this in mind, the p*K*<sub>a</sub> of residues *n* to *n* + 3 in each alternate tetramer was set to 9, resulting in a protonated carboxylic acid. Each simulation took place inside a simulation box filled with water molecules and Na<sup>+</sup> and Cl<sup>-</sup> ions were added to neutralize the system to a pH of 7.4 with constant pressure and a temperature of 298 K. All structures were initially energy-minimized without electrostatic interactions by a short steepest descent minimization. After removal of conformational stress, simulated annealing was performed (time step 2 fs, atom velocities scaled down by 0.9 every 10th step) until convergence was reached, that is, the energy improved by <0.05 kJ mol<sup>-1</sup> atom<sup>-1</sup> during 200 steps (see Experimental for additional details).

An encouraging result of this initial treatment was the adoption by PIAA(A) of a more isotropic, smoothly cylindrical organization (Figure 1D). To determine its practical average diameter, the distance between carboxylate atoms in directly opposite pairs was measured at several points along the polymer (see Supporting Information Table S3), revealing diameters of 16.1 Å (PIAA) and 17.3 Å (PIAAA). For comparison, the DNA provided by PDB 3U5Z spans 21.8 Å. It is known that a layer of water lubricates the DNA-protein interaction,<sup>[30]</sup> suggesting that average diameters are not as influential as they appear at first. As shown in the Supporting Information, the structure is stable, with negligible atom mobility in its interior atoms compared to a sway of ~5 Å for the residue-terminating carboxyl oxygen. Nonetheless, this initial approach hints at PIAAA as being the more promising candidate for interacting with the gp45 clamp.

To visualize the possible interactions between PIAAA as a synthetic template and the set of gp45 clamp and gp44/62 clamp loader proteins, the energy-minimized model of PIAAA was manually placed inside a complex of the gp45 clamp and gp44/62 clamp loader proteins.<sup>[31]</sup> Figure 3 shows perspectives on the clamp protein as it interacts with DNA in a crystal structure (PDB 3U5Z) and as it might similarly interact with PIAAA. These modelling outcomes prompted a more experimental approach for assessing the capacity of PIAA(A) to function as a template.

#### 3.2 | Binding studies

As discussed earlier, a dedicated clamp loader protein complex exists, that is, gp44/gp62, which opens the clamp protein and transfers it onto the substrate. This clamp loading activity can be monitored through an ATPase assay following published procedures.<sup>[7,14]</sup> A schematic outline for this assay is provided in section 2 Experimental (see above). The clamp loader complex consumes four ATP molecules per



**FIGURE 3** Clamp (gp45, left) and clamp-loader (gp44/62, right) model (3U5Z) with manual placement of PIAAA

successful loading event: two ATP molecules are hydrolysed to close the clamp on a template (which in Nature is DNA) and two more are consumed when the gp44/62 complex dissociates.<sup>[32,33]</sup> In the experimental setup, gp45 and gp44/62 are equilibrated before a polymer template is added. In this equilibrium, the clamp and the clamp loader complex associate. When ATP hydrolysis is measured in a coupled spectrophotometric assay only background ATP hydrolysis is detected when no template is present. The background ATPase rate originates from chemical hydrolysis, or from the new recruitment of a gp45 clamp by the clamp loader after a dissociation event has taken place (see Table 1, entry for +gp45). Upon addition of a polymer template, a change in the ATP hydrolysis rate is measured, resulting not only from the loading of the pre-associated clamps upon said template, but also from the increased rate with which new clamps are reloaded, after dissociation from a template. In this assay, an increase in ATP consumption is only observed when all components of the system are present, including a suitable polymer template. This provides evidence towards the efficacy of this system for detecting actual clamp loading events on both natural and unnatural polymeric templates.

We performed ATPase assays using a variety of polymeric templates, that is, a series of DNA templates, PIAA, PIAAA, and poly(ethylene imine) (PEI). The latter polymer was included as a negative control, since PEI is highly cationic, has a small persistence length, and no helicity leading one to consider it not to be a suitable template polymer. The DNA templates that were chosen include a forked DNA substrate, which mimics the natural loading site of gp45,<sup>[15]</sup> as a positive control, and a freshly prepared supercoiled plasmid as a negative control. The 5'-Overhang DNA, blunt-ended DNA, and nicked circular DNA were also compared. These substrates were chosen for their diversity and to provide a context for the measurements with the synthetic polymers.

Table 1 reports the ATPase results, which include the background gp44/62-gp45 rates, and the rates after addition of the templates. Not surprisingly, the ideal Fork substrate presented an increase of well over 700%. This substrate is short and acts as a model for the natural DNA loading site. Its short length makes that the clamp loads easily, but also

unloads rapidly, explaining the high rate. More interesting are the results for the other DNA templates: supercoiled and nicked plasmid elicited a decrease of over 20% in ATPase rate, suggesting that the presence of these templates shifts the equilibrium balance of the

**TABLE 1** T4 clamp loading ATPase rates on different DNA and polymer templates

Enzyme components	ATPase nM s <sup>-1a</sup>		
Blank <sup>b</sup>	8.0–26 <sup>b</sup>		
+gp44/62	4.1 ± 0.6		
+gp45	57 ± 9		
DNA species	ATPase nM s <sup>-1a</sup>	% Change <sup>c</sup>	Significance <sup>d</sup>
Fork	501 ± 40	771%	***
Supercoiled	46 ± 3.4	-21%	*
Nicked	42 ± 1.7	-26%	**
Blunt	62 ± 2.8	8%	—
5' overhang	150 ± 9	161%	**
Polymer species	ATPase nM s <sup>-1a</sup>	% Change <sup>c</sup>	Significance <sup>d</sup>
PIAA	59 ± 4.9	3%	—
PIAAA	72 ± 6.0	26%	**
PIAAA blank	13 ± 8.0 <sup>e</sup>		
PEI	45 ± 3.9	-21%	*

<sup>a</sup>ATPase activities (± standard error).

<sup>b</sup>Blank reaction is the activity of the starting solution (buffer with ATP, phosphor-(enol)-pyruvate, NADH, LDH/PK) without any gp44/62, gp45, DNA or synthetic polymer present. It varies in each of the separate experiments and is subtracted from the actual rates. The numbers in the table have been corrected for this blank reaction.

<sup>c</sup>Calculated percent change from gp44/62/45 rate.

<sup>d</sup>Calculated significance compared to gp44/62/45 ATPase (*P* values: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001).

<sup>e</sup>Blank reaction in which only PIAAA and no enzymes +gp45 and +gp44/62 were added.

clamp-clamp loader complex downwards, either through more stable loading, or through nonproductive sequestering interactions with the templates. Interestingly, loading onto a nicked double-stranded DNA had been previously identified as a viable loading site using various techniques,<sup>[15]</sup> and was even observed on a single-molecule basis.<sup>[7]</sup> Blunt DNA and 5'-overhang DNA showed stimulations in ATPase rates of 8 and 161%, respectively, which demonstrates preferential loading at a 3' primer over nonoverhangs. The preferential suitability of 5'-overhang DNA is not surprising, because clamps are known to be loaded there during leading and lagging strand replication.<sup>[10,34]</sup>

The synthetic polymers provided the following results. The negative control PEI showed a negative change in ATPase rate, highly similar to that of the negative control supercoiled plasmid. An actual increase in the ATPase rate, albeit small (3%), was observed for PIAA. More importantly, PIAAA exhibited an increase in ATPase rate of 26% ( $P < 0.01$ ), which is more than three times the increase shown by a blunt DNA template, and about one sixth of the activity elicited by a 5'-overhang DNA template. It should be noted, however, that the PIAAA polymer itself, that is, without gp45 and gp44/62 added, also evoked an effect, albeit a much smaller one (see Table 1, row 11). Nevertheless, this result indicates that a synthetic polymer is capable of stimulating the loading activity of a sliding clamp protein and its clamp loader complex. These experimental results are consistent with the molecular dynamics modelling of PIAA and PIAAA, and the subsequent identification of PIAAA as a possible candidate template based on its apparent diameter, peripheral anionic sites, persistence length, and helicity.

It is interesting to note that dsDNA, of course, has even more DNA-like persistence length, helicity, and charge distribution. Still, Table 1 confirms the complete absence of clamp loading induced by supercoiled or nicked dsDNA, whereas loading on PIAAA stimulates the ATPase rate. This is not surprising, because the clamp-loader complex has evolved to be selective in clamp-loading at sites of replication or repair. Our results suggest that PIAAA is a suitable, but certainly not a perfect template for clamp loading. If PIAAA had resembled double-stranded DNA too closely, it might not have promoted the activity of the clamp loader as it did.

A final consideration is that the PIAA(A) polymers used in this study are linear. This in itself is not surprising, but it contrasts features of the circular plasmid and the nicked DNA template, and, to a certain extent, the fork substrate. It could be the case, therefore, that the clamp loader recognizes the polymer terminus and loads the clamp there, partially denying the influence that the further properties of PIAA(A) might have. However, in this case the suitability of PIAAA would be even more impressive, since blunt DNA is linear as well, while its increase in ATPase rate is similar to that of PIAAA. Also, PIAA(A) is homogeneous over its entire length and thus would offer a stretch of potential loading sites if loading did not occur at termini, whereas the DNA templates mostly offer discrete loading loci at their overhang or nick. As such, the blunt DNA appears to be the DNA template that most closely matches the PIAA(A) templates in their features. Synthesis of a circular PIAA(A), or of a PIAA(A) where both termini are sterically blocked, would allow the experimental exclusion of a terminus effect,

but this is synthetically very demanding. One of our future goals is to visualize the PIAAA/protein complexes to further investigate the specific location of the loading event.

## 4 | CONCLUSIONS AND OUTLOOK

We have subjected helical, polyanionic, and rigid polyisocyanides to molecular modelling, and it was found that the synthetic polymers PIAA(A) adopt a cylindrical conformation, sterically resembling DNA to the extent that it would fit inside a gp45 clamp protein. Enzyme-coupled ATPase assays were performed to investigate the activity of the gp44/62 clamp loader while it loads the clamp onto various templates. The template PIAAA was found to induce a 26% increase in clamp loading activity, exceeding that of blunt DNA, which is the best natural analogue.

The experiments described in this article were performed to see whether synthetic polymers might be used in combination with clamp proteins to construct processive catalytic systems. Such bio-hybrid systems are of interest because they may open a route to encode synthetic polymers with chemical information. Experiments in which DNA is encoded with information by a step-wise synthesis protocol have already been reported in the literature.<sup>[35]</sup> Nature, however, makes use of catalytic systems, that is, polymerases to copy and store information. Synthetic catalytic systems capable of doing this are very rare<sup>[2]</sup> and it would be of great interest to develop them. Given the outcome of our experiments we may conclude that synthetic polymers, that is, PIAAA, may be used in combination with suitably modified clamp proteins, but the resulting systems will not be very robust because the interaction between the synthetic polymer and the clamp protein is not strong enough for data encoding. For data storage purposes it may be better to keep DNA as the encoding template and use it in combination with a gp45 clamp that is coupled to a suitable DNA modifying enzyme, for example, a C-5 cytosine-specific DNA methylase or a related enzyme. Current work is aimed at developing such bio-catalytic DNA-encoding systems.

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## AUTHOR CONTRIBUTIONS

R.J.M. Nolte and S.F.M. van Dongen designed the experiments. O.I. van den Boomen, M. Pervaiz, and T. Ritchel performed the calculations. J. Clerx, M.A. Trakselis, D.C. Schoenmakers, and L. Schoonen

carried out the experiments. S.F.M. van Dongen and R.J.M. Nolte wrote the article.

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