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14. ABSTRACT This report summarizes our progress in the STIR funded 9 month period in developing magnetic tweezers based approach to visualize the second-generation Grubbs catalyst (G2) catalyzed ring opening metathesis polymerization (ROMP) at the single-polymer level in real time. Toward this goal, we have made the following progresses: (1) Modification of the G2 catalyst for surface linkage to the magnetic particle (or glass slide). (2) Set-up and testing of the MT instrument. (3) Manipulation of polynorbornene molecules. (4) Real-time ROMP of norbornene under no force load. These results provide the foundation and demonstrate the feasibility of using magnetic tweezers to					
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Report Title

Final report - STIR: Single-molecule visualization of living polymerization

ABSTRACT

This report summarizes our progress in the STIR funded 9 month period in developing magnetic tweezers based approach to visualize the second-generation Grubbs catalyst (G2) catalyzed ring opening metathesis polymerization (ROMP) at the single-polymer level in real time. Toward this goal, we have made the following progresses: (1) Modification of the G2 catalyst for surface linkage to the magnetic particle (or glass slide). (2) Set-up and testing of the MT instrument. (3) Manipulation of polynorbornene molecules. (4) Real-time ROMP of norbornene under no force load. These results provide the foundation and demonstrate the feasibility of using magnetic tweezers to follow and manipulate single-polymer growth during ROMP by a single catalyst molecule.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

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Number of Presentations: 0.00

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Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

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Number of Manuscripts:

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Patents Submitted

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2014 ACS PHYS Division Early-Career Award in Experimental Physical Chemistry

2014 Coblentz Award

2013 Honorable Lecturer, Applied Chemistry Lecture Series, CIAC, Chinese Academy of Sciences

2013 Peter J. W. Debye Professor of Chemistry

2013 Lester S. Andrews Lecturer, Mississippi State University

Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	Discipline
Ace Santiago	0.17	
Feng Yang	0.17	
Guanqun Chen	0.25	
Ningmu Zou	0.25	
Kaori Kubo	0.05	
FTE Equivalent:	0.89	
Total Number:	5	

Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
Chunming Liu	0.05
FTE Equivalent:	0.05
Total Number:	1

Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	National Academy Member
Peng Chen	0.00	
FTE Equivalent:	0.00	
Total Number:	1	

Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
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Inventions (DD882)

Scientific Progress

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Technology Transfer

Proposal #: 62469CHII

Agreement #: W911NF1310043

Title: STIR: Single-molecule visualization of living polymerization; Area 7.3 Polymer Chemistry

PI: Peng Chen, Department of Chemistry and Chemical Biology, Cornell University

Final Report: Scientific Progress and Accomplishments (03/15/2013–12/14/2013)

We have proposed to use a *magnetic tweezers (MT) approach to visualize real-time single-polymer growth to study polymerization catalysis*. **Fig 1A** illustrates our experimental design of using MT to monitor the length of the polymer in real time while it grows from a single catalyst molecule. We propose to tether the polymer between a glass surface and a magnetic particle, and its growth will be catalyzed by a single transition metal catalyst, which is part of the polymer tether and can be attached to either the particle or the glass slide side (**Fig 1B**). The magnets exert an upright, and variable, force on the magnetic particle to stretch the polymer. By monitoring the vertical (z) position of the particle in real time under catalysis, we can monitor the length extension rate, which reflects the polymerization kinetics at the catalyst. Moreover, by varying the applied force (via changing the magnet position), we can also probe how mechanical tension affects the catalytic kinetics of the polymerization. When the magnets are positioned far away, we can also reach zero force load for the polymerization reaction; here, the particle will undergo thermally driven, but tether-constrained, Brownian motion, and its horizontal mean square displacement $\langle \Delta x^2 \rangle$ or $\langle \Delta y^2 \rangle$ is directly related to the length of the polymer¹ and can thus be used to report the polymer growth as well.

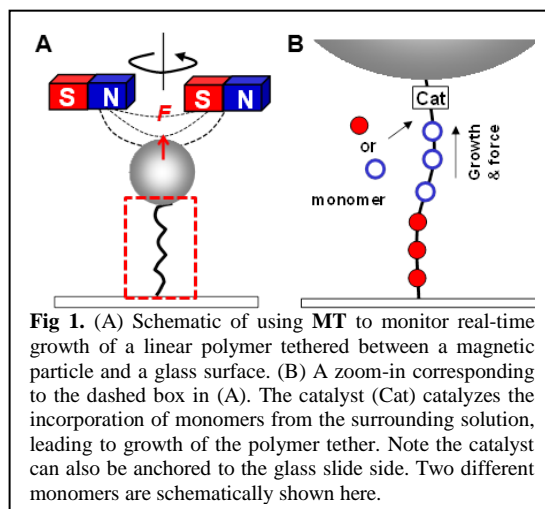


Fig 1. (A) Schematic of using MT to monitor real-time growth of a linear polymer tethered between a magnetic particle and a glass surface. (B) A zoom-in corresponding to the dashed box in (A). The catalyst (Cat) catalyzes the incorporation of monomers from the surrounding solution, leading to growth of the polymer tether. Note the catalyst can also be anchored to the glass slide side. Two different monomers are schematically shown here.

This MT approach offers many features and advantages: (1) Polymerization kinetics can be determined directly from the rate of polymer end-to-end extension, which is related to the polymer contour length using several theoretical models, such as the worm-like-chain model, freely jointed chain model, and freely rotating chain model^{2,3}. (2) The wide-field imaging format in MT allows for high throughput measurements of many individual polymers growing in real time. (3) If different monomers are incorporated with distinct kinetics or distinct unit-length extension, microstructure sequencing of a single copolymer becomes possible. (4) By rotating the magnets, we can apply a torsional force, which allows for the differentiation of single-polymer or multi-polymer tethers between the particle and the surface.

Our initial model system is the ring-opening metathesis polymerization (ROMP) catalyzed by the 2nd generation Grubbs catalyst (G2 catalyst, **Fig 2A**), for its well-known reaction mechanism, robustness of the catalyst, as well as availability of various monomers and catalyst derivatives^{4,5}. For monomers, we propose to

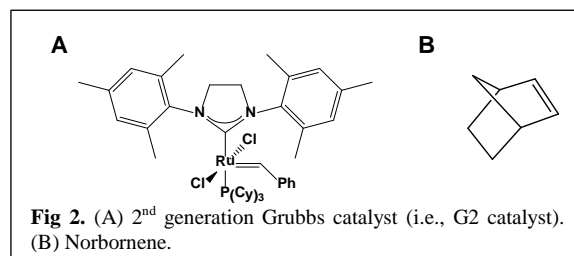


Fig 2. (A) 2nd generation Grubbs catalyst (i.e., G2 catalyst). (B) Norbornene.

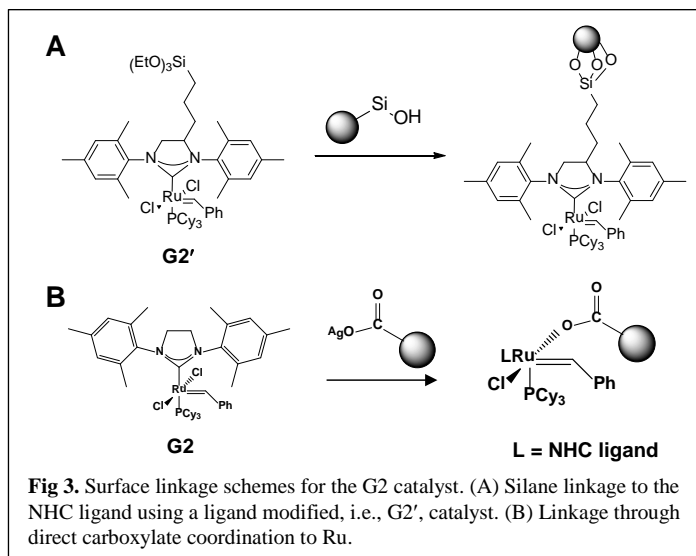
initially use norbornene as our *model monomer* for the polymerization (**Fig 2B**).

Based on the above experimental design and model catalyst and monomer, we have made the following *progresses* in the 9-month STIR funding period:

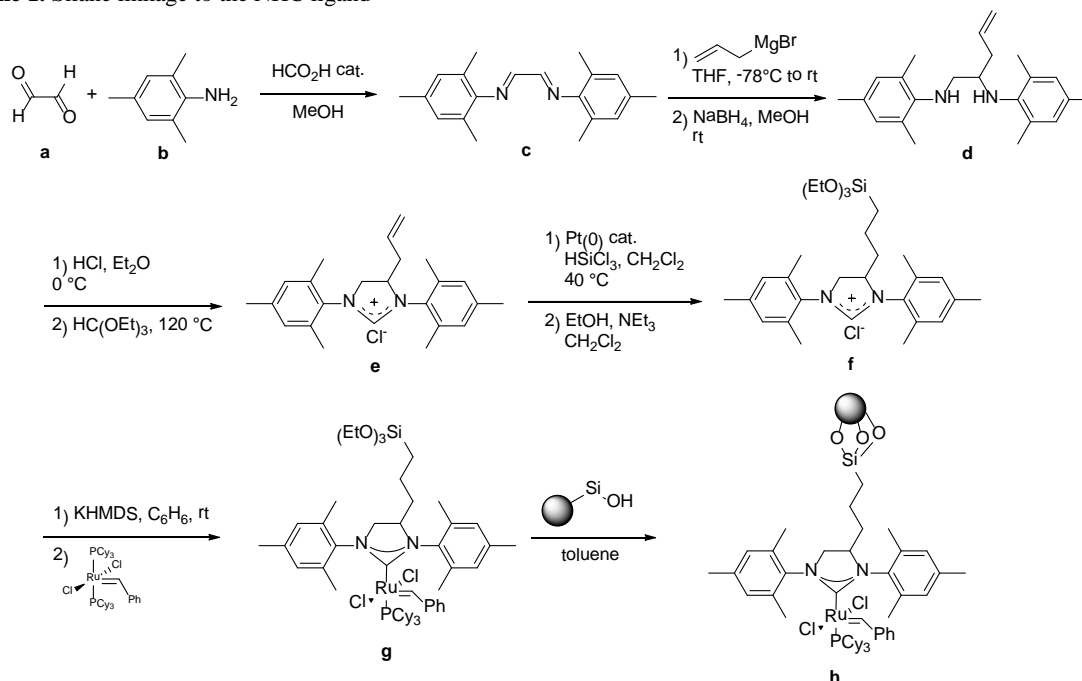
1. Modification of the G2 catalyst for surface linkage to the magnetic particle (or glass slide)

We use polystyrene magnetic particles of 1-3 μm in diameter, as typically used in MT experiments⁶⁻⁹, with two types of surface functional groups: carboxylate or silanol. Both of these particles are available from Invitrogen (Dynabeads® MyOne™). These magnetic particles have excellent size uniformity (<3% size dispersion). Their surface functional groups will allow for linkage to the G2 catalyst that will also be modified for linkage (see below).

We have designed two different schemes to link the G2 catalyst to the magnetic particle. The first scheme uses an ethoxysilane modified N-heterocyclic carbene (NHC) ligand of the G2 catalyst for linkage to silanol-coated particle (**Fig 3A**). This ligand modification scheme retains the coordination environment of the Ru center and thus its catalytic activity. We have followed established procedures (**Scheme 1**)^{10,11} to make the modified NHC ligand and obtained the pure compound (compound **f** in **Scheme 1**,



Scheme 1. Silane linkage to the NHC ligand

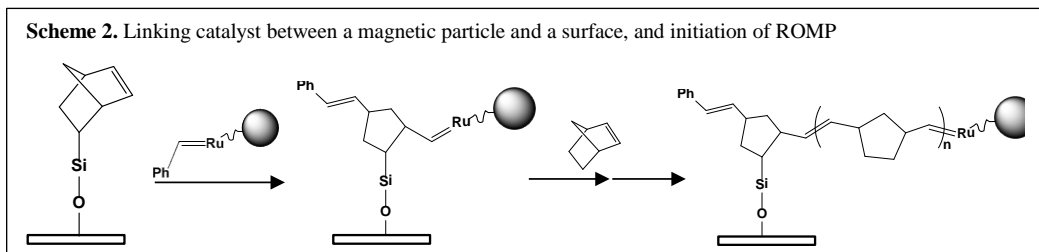


NMR characterized). We have also exchanged the modified NHC onto the first generation Grubbs catalyst to generate the product G2' (compound **g** in **Scheme 1**) and currently are working on its purification.

The second scheme involves converting the carboxylate functional groups on the magnetic particle into silver-carboxylates, which can then be attached to the Ru center of the G2 catalyst via substitution of a chloride ligand (**Fig 3B**)^{12,13}. The advantage of this scheme is its simplicity, but it modifies the Ru coordination environment, which can change the catalyst activity.

Once the catalyst is linked to the particle, it can be linked to a surface-grafted

norbornene group (**Scheme 2**), after which norbornene can be added to initiate the ROMP reaction for measurement.

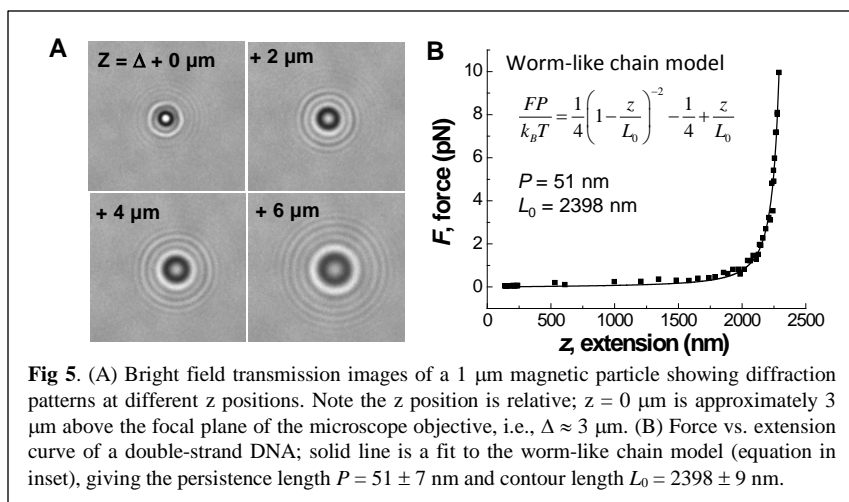
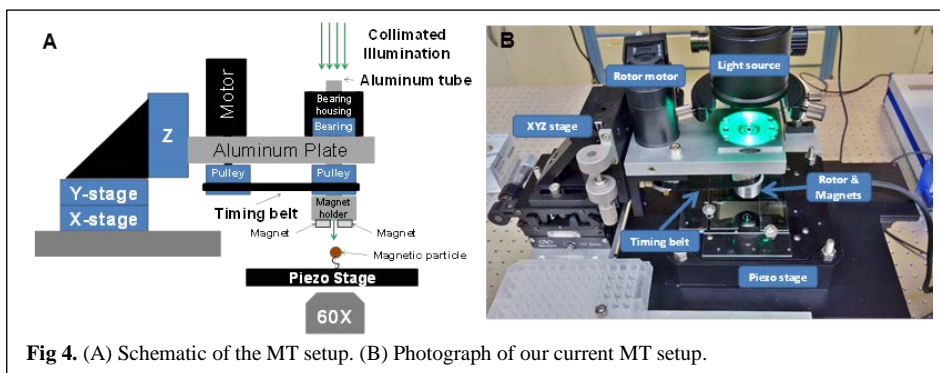


2. Set-up and testing of the MT instrument

We have built a MT on top of an Olympus IX71 inverted microscope, following the design of Neuman *et al.*¹⁴ (**Fig 4**). The major components of the setup include: two rectangular NdFeB magnets mounted on a rotatable base controlled by a motor via a timing belt, collimated LED illumination, xyz control

of the magnet position in μm precision, xyz control of the sample position in nm precision via a piezo stage, a high magnification objective, and a camera capable of 200 Hz frame rate.

We have used this MT set up to measure force vs. extension curves of single immobilized double-strand DNA of known length

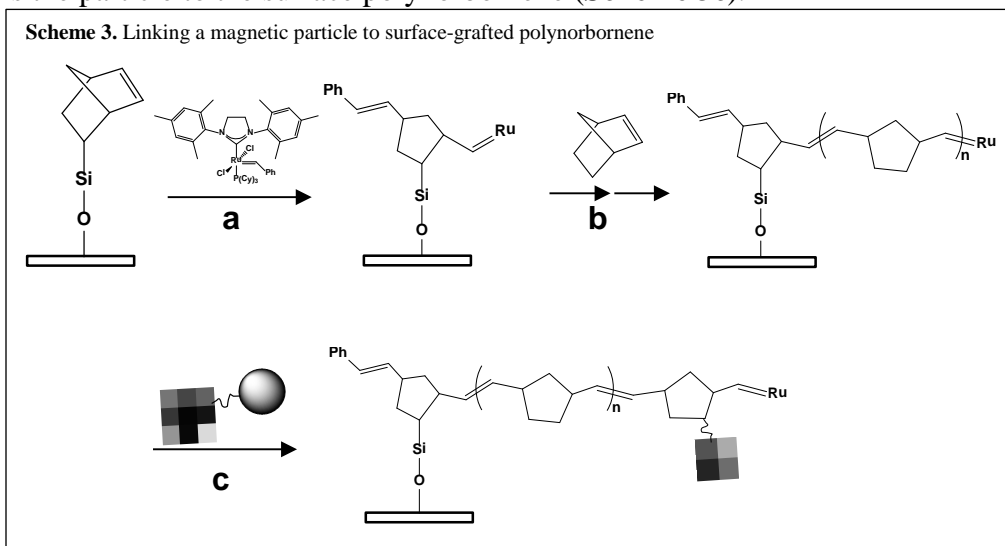


to test and calibrate our new instrument. **Fig 5A** shows the bright-field transmission images of a DNA-tethered 1- μm magnetic particle at different z positions, which are determined by analyzing the optical diffraction patterns¹⁵ coupled with calibration with known z positions. The x and y positions of the particle are determined by tracking the center of its image. The motions of the magnetic particle in the xy plane also report the vertical magnetic force (F) through the fluctuation-dissipation theorem: $F = k_B T l / \langle \Delta x^2 \rangle$, where k_B is the Boltzmann constant, T is temperature, l is the tether end-to-end extension distance, and $\langle \Delta x^2 \rangle$ is the transversal position variance of the particle¹⁶. The end-to-end distance l is obtained by measuring the z position of the particle relative to a marker particle stuck to the glass surface. The measured force-vs.-extension curve in **Fig 5B** behaves as expected^{14,15} and is well fitted with a worm-like chain model, which gives the DNA contour length (L_0) and persistence length (P).

3. Manipulation of polynorbornene molecules

We have also tried our MT setup to manipulate linear polynorbornene molecules grown on surfaces. In this preliminary experiment, we started with a norbornene-functionalized glass surface and reacted with the original G2 catalyst, which undergoes a ring opening metathesis and gets linked to the surface (**Scheme 3a**). We then flowed in norbornene to initiate ROMP to grow polynorbornene for ~ 1 hour (**Scheme 3b**) — *all polymer experiments were carried out in dry and degassed toluene solvent*. After washing out excess norbornene, we added the 1- μm magnetic particles whose surface was pre-functionalized with norbornene via silane chemistry. The norbornene on the particle surface gets inserted at the end of surface polynorbornene, and at the same time, links the particle to the surface polynorbornene (**Scheme 3c**).

Using these polynorbornene-tethered magnetic particles on the surface, we used our MT setup to mechanically stretch the polymer. **Fig 6A** shows two force vs. extension curves from two tethered



magnetic particles, which show very different extension lengths over the same applied force range. These different extension lengths reflect the heterogeneity in chain length among the surface polynorbornene molecules; *this heterogeneity is ubiquitous in polymer synthesis, for which our MT-based measurements offer advantages in probing one polymer chain at a time*. The force vs. extension of the shorter polynorbornene can be satisfactorily fitted by the worm-like-chain model (WLC) (hollow red squares, **Fig 6A**), giving the persistence length $P = 0.92 \pm 0.07$ nm and contour length $L_0 = 912 \pm 8$ nm. This P is reasonable for a single polynorbornene chain in toluene (a good solvent for polynorbornene), considering the P of a single PEG chain in

water is 0.47 nm measured by MT (water is a good solvent for PEG)¹⁷. In contrast, the longer polynorbornene in **Fig 6A** (solid black circles) cannot be sufficiently fitted by the WLC model over the entire force-extension curve; an *attempted* fit gives the apparent persistence length $P = 1.40 \pm 0.09$ nm and contour length $L_0 = 5360 \pm 100$ nm. The much larger apparent persistence length suggests that this polymer tether is much stiffer and thus this magnetic particle is possibly tethered by multiple polymer chains.

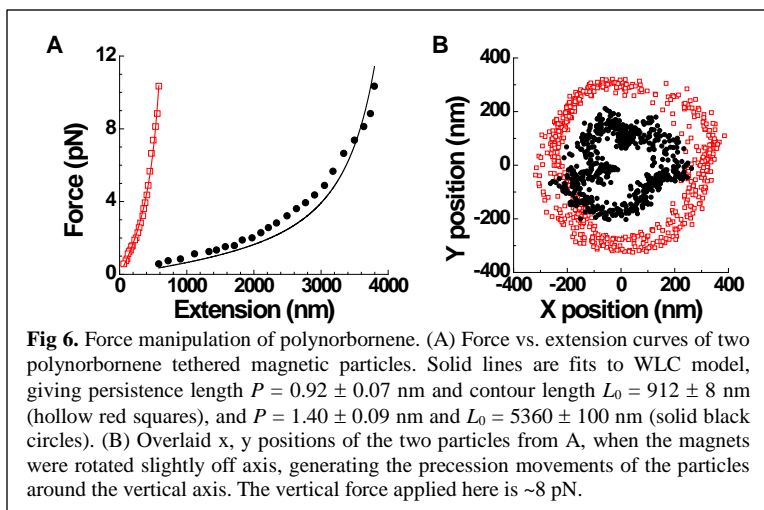


Fig 6. Force manipulation of polynorbornene. (A) Force vs. extension curves of two polynorbornene tethered magnetic particles. Solid lines are fits to WLC model, giving persistence length $P = 0.92 \pm 0.07$ nm and contour length $L_0 = 912 \pm 8$ nm (hollow red squares), and $P = 1.40 \pm 0.09$ nm and $L_0 = 5360 \pm 100$ nm (solid black circles). (B) Overlaid x, y positions of the two particles from A, when the magnets were rotated slightly off axis, generating the precession movements of the particles around the vertical axis. The vertical force applied here is ~ 8 pN.

We further rotated the magnets to generate torsional force while the rotation axis was slightly off the vertical axis. At the applied vertical force of ~ 8 pN when the short and long polymer tethers are at ~ 530 nm and 3600 nm extensions, respectively (**Fig 6A**), this magnet rotation generates precession movements of the particles around their respective vertical axes. **Fig 6B** shows the x and y positions of the two polynorbornene-tethered particles from **Fig 6A**. The *shorter*-polynorbornene-tethered particle shows free precession movement with its x , y positions following a circular pattern with a diameter of ~ 250 nm (hollow red squares). In contrast, the *longer*-polynorbornene-tethered particle shows restricted precession movement where its x , y positions do not follow well a circular pattern (solid black circles). Furthermore, the radius of its restricted precession is smaller, ~ 120 nm, even though this tether is significantly longer. This restricted precession again suggests that this particle is likely tethered by multiple polynorbornene chains¹⁷.

These results demonstrate the feasibility of using our MT setup to mechanically manipulate polynorbornene-tethered magnetic particles and differentiate single vs. multiple polymer tethers. This type of force vs. extension curves will also provide the basis for analyzing the extension lengths of single polynorbornene chains to obtain their contour lengths, whose real-time changes will report the polymerization catalysis kinetics in situ.

4. Real-time ROMP of norbornene under no force load

We have also tried following single-molecule ROMP of norbornene in real time under no force load, i.e., the magnets were positioned far away from the surface or using non-magnetic particle (reference **Fig 1**). In this preliminary experiment, we linked the magnetic particle to the surface-immobilized G2 catalyst via carboxylate coordination to Ru as in **Fig 3B**. The catalyst was immobilized via reaction with norbornene-functionalized glass surface as in **Scheme 3a**. **Fig 7A** shows the x -position versus time of a surface-tethered particle under ROMP reaction conditions (~ 1 M norbornene in toluene). The constrained Brownian motion of the particle is clear from the oscillating x -positions, and the Brownian motion amplitude increases gradually, reflecting the growing length of the polymer tethered between the particle and the quartz surface. The corresponding cumulative mean square displacement, $\langle \Delta x^2 \rangle$, increases with time, as expected, again reflecting the underlying real-time polymer growth (**Fig 7B**). The y -position and

$\langle \Delta y^2 \rangle$ behave similarly versus time. A note of caution here – we do not yet know for sure that in this case there are only one polymer and one catalyst tethered between the particle and the quartz surface. But this can be probed using the torsional manipulation as in Fig 6B.

As a control, we also performed similar imaging experiments on the same type of particles that were adsorbed on norbornene functionalized quartz surface that does not contain the G2 catalysts. Fig 7C shows the x -position trajectory of such a particle. This particle is essentially stationary, except for the measurement noise, in sharp contrast to that in Fig 7A; and no increase in the noise level was observed over a same period of time either. Correspondingly, the calculated cumulative mean square displacement, $\langle \Delta x^2 \rangle$, stays essentially flat with time (Fig 7D). The y -position trajectory and $\langle \Delta y^2 \rangle$ behave similarly. These results support the feasibility of real-time visualization of ROMP using our approach.

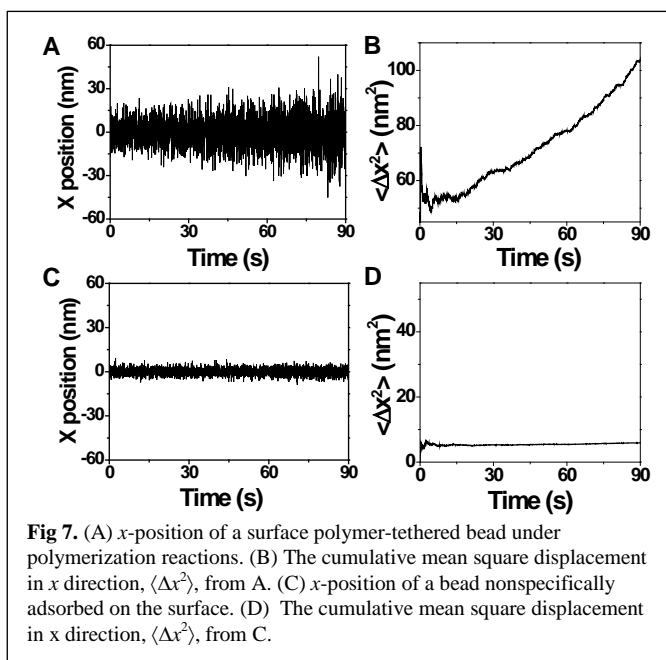


Fig 7. (A) x -position of a surface polymer-tethered bead under polymerization reactions. (B) The cumulative mean square displacement in x direction, $\langle \Delta x^2 \rangle$, from A. (C) x -position of a bead nonspecifically adsorbed on the surface. (D) The cumulative mean square displacement in x direction, $\langle \Delta x^2 \rangle$, from C.

Bibliography

- 1 H. Qian & E. L. Elson. Quantitative study of polymer conformation and dynamics by single-particle tracking. *Biophys J* **76**, 1598-1605 (1999).
- 2 J. Liang & J. M. Fernández. Mechanochemistry: One bond at a time. *ACS Nano* **3**, 1628-1645 (2009).
- 3 P. Schwaderer, E. Funk, F. Achenbach, J. Weis, C. Brauchle & J. Michaelis. Single-molecule measurement of the strength of a siloxane bond†. *Langmuir* **24**, 1343-1349 (2007).
- 4 M. Scholl, S. Ding, C. W. Lee & R. H. Grubbs. Synthesis and activity of a new generation of ruthenium-based olefin metathesis catalysts coordinated with 1,3-dimesityl-4,5-dihydroimidazol-2-ylidene ligands. *Org Lett* **1**, 953-956 (1999).
- 5 G. C. Vougioukalakis & G. R. H. Ruthenium-based heterocyclic carbene-coordinated olefin metathesis catalysts. *Chem Rev* **110** (2010).
- 6 T. Lionnet, J. F. Allemand, A. Revyakin, T. R. Strick, O. A. Saleh, D. Bensimon & V. Croquette. Single-molecule studies using magnetic traps. *Cold Spring Harb Protoc* **2012**, 34-49 (2012).
- 7 I. D. Vilfan, J. Lipfert, D. A. Koster, S. G. Lemay & N. H. Dekker. in *Handbook of Single-Molecule Biophysics* (ed A. v. O. P. Hinterdorfer) (Springer Science+Business Media, LLC, 2009).
- 8 Y. Seol & K. C. Neuman. Magnetic tweezers for single-molecule manipulation. *Methods Mol Biol* **783**, 265-293 (2011).

- 9 I. De Vlaminck & C. Dekker. Recent advances in magnetic tweezers. *Annu Rev Biophys* **41**, 453-472 (2012).
- 10 X. Bantreil & S. P. Nolan. Synthesis of n-heterocyclic carbene ligands and derived ruthenium olefin metathesis catalysts. *Nat Protoc* **6**, 69-77 (2011).
- 11 D. P. Allen, M. M. Van Wingerden & R. H. Grubbs. Well-defined silica-supported olefin metathesis catalysts. *Org Lett* **11**, 1261-1264 (2009).
- 12 T. S. Halbach, S. Mix, D. Fischer, S. Maechling, J. O. Krause, C. Sievers, S. Blechert, O. Nuyken & M. R. Buchmeiser. Novel ruthenium-based metathesis catalysts containing electron-withdrawing ligands: Synthesis, immobilization, and reactivity. *J Org Chem* **70**, 4687–4694 (2005).
- 13 W. Buchowicz, A. Makal & K. Woźniak. The bis(trifluoroacetate) analogue of the first-generation grubbs catalyst: Synthesis, x-ray structure, and metathesis activity of $[\text{Ru}(\text{CF}_3\text{CO}_2)(\eta^2\text{-CF}_3\text{CO}_2)(\text{chph})(\text{pcy}_3)_2]$. *J Organometal Chem* **694**, 3179–3183 (2009).
- 14 Y. Seol & K. Neuman. in *Single molecule analysis* Vol. 783 (eds E. J. G. Peterman & G. J. L. Wuite) 265 (Humana Press, 2011).
- 15 A. J. W. te Velthuis, J. W. J. Kerssemakers, J. Lipfert & N. H. Dekker. Quantitative guidelines for force calibration through spectral analysis of magnetic tweezers data. *Biophys J* **99**, 1292–1302 (2010).
- 16 A. M. van Oijen, P. C. Blainey, D. J. Crampton, C. C. Richardson, T. Ellenberger & X. S. Xie. Single-molecule kinetics of λ exonuclease reveal base dependence and dynamic disorder. *Science* **301**, 1235-1238 (2003).
- 17 A. Dittmore, D. B. McIntosh, S. Halliday & O. A. Saleh. Single-molecule elasticity measurements of the onset of excluded volume in poly(ethylene glycol). *Phys Rev Lett* **107**, 148301 (2011).