

ChemComm

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

COMMUNICATION

A molecular “screw-clamp”: accelerating click reactions in miniemulsions

Cite this: DOI: 10.1039/x0xx00000x

Evandro M. Alexandrino^a, Philipp Buchold^a, Manfred Wagner^a, Adrian Fuchs^a, Andreas Kreyes^a, Clemens K. Weiss^{a,b}, Katharina Landfester^a and Frederik R. Wurm^{a,*}

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

The interface as a “screw clamp”: the copper-free 1,3-dipolar azide-alkyne cycloaddition at the interface of nanodroplets in miniemulsions was studied in detail by NMR spectroscopic methods. The reaction at the oil-water interface proved to exhibit higher rate constants, increased molecular weights and high regioregularity compared to the reaction in solution.

The 1,3-dipolar Huisgen cycloaddition between alkynes and azides (AAC) is certainly the most successful and widespread type of ‘click’ reactions resulting in the formation of 1,2,3-triazoles.¹ The reaction developed by Huisgen was originally a thermally activated relatively slow reaction, which generated a mixture of two regioisomers: 1,4- and 1,5- disubstituted 1,2,3- triazoles.² Later, Sharpless and coworkers³ and Medal and coworkers⁴ obtained regioselective products (1,4-disubstituted 1,2,3- triazoles) with high yields by the use of copper catalysts at mild conditions. Ruthenium catalysts have also been used for the preparation of 1,5-disubstituted 1,2,3 triazoles⁵ and recently iridium for the preparation of regioselective products from electron rich internal alkynes.¹ The removal of these metal catalysts however, can be challenging especially for biological applications.^{6, 7} Some approaches to metal-free AAC have been successfully developed including strained cyclooctynes,^{7, 8} activated alkynes^{7, 9} and electron deficient alkynes.^{7, 10, 11} Concerning the various alkyne derivatives, the electron deficient propiolates are of special interest due to the straightforward esterification of the alcohol of choice with propiolic acid.¹⁰ The absence of a catalyst and the bioorthogonal functional groups make this reaction highly suitable for the preparation of materials for biomedical applications, e.g. the formation of bioconjugates, nanocarriers, or biodegradable surfactants. To obtain reasonable reaction rates however, elevated temperatures are still necessary and which may for example, denature biomolecules. Another interesting aspect of this reaction is the feasible combination of two immiscible

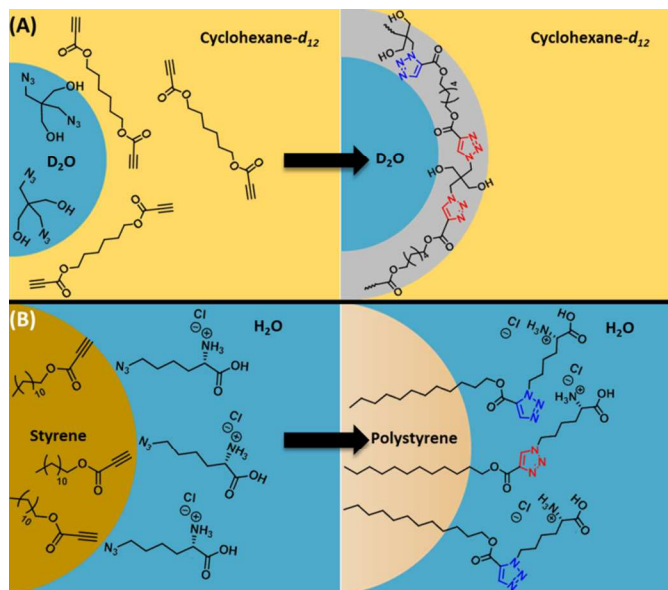
reactants via an interfacial click reaction to either generate a polymeric shell or an amphiphilic material like a surfactant, via orthogonal chemistry.

This work explores the possibilities of the copper-free click reaction between azides and electron deficient propiolates at the droplet interface of a stable miniemulsion. First, we investigate the effect on reaction kinetics of an A₂ + B₂ polyaddition with respect to conversion and molecular weights. The resulting polyesters are also compared to solution protocols at different temperatures. Further, the droplet interface is used to generate a surfactant via an *in situ* click reaction. The resulting stabilized miniemulsion could be further employed in the radical polymerization of styrene. In the miniemulsion, droplets with a very high total surface area in comparison to conventional emulsions or a homogeneous reaction system are generated.¹² These monodisperse droplets can be used as nanoreactors for reactions inside or at the interface of the droplets.¹²

The kinetics of the reaction and the structural features of both systems were characterized in detail by various NMR spectroscopic techniques and gel permeation chromatography (GPC). Previously, our group has successfully developed a facile method to produce nanocapsules by copper-free click polymerization for encapsulation of functional molecules using a water soluble azide (2,2-bis(azidomethyl)propan-1,3-diol, BAP) and an organic soluble alkyne (hexane-1,6-diyl dipropiolate, HDDP).¹³ Following from these results, the detailed kinetic investigation of the copper-free click reaction at the interface of a miniemulsion was studied. Further, the polymerization of HDDP and BAP at the interface and the use of this methodology to synthesize amphiphilic molecules, like surfactants, was also achieved (Scheme 1).

¹H NMR spectroscopy was used for the *in situ* measurement of the polymerization kinetics¹⁴ over a period of 800 min for the various reactions. Both solution and inverse miniemulsion polymerizations were carried out in either DMSO-*d*₆, or cyclohexane-*d*₁₂ as continuous phase and D₂O as dispersed phase respectively.

Temperatures of 298 K and 323 K with a delay time of approximately 8 min between each spectral acquisition were used (see Supporting Info Fig S3-S4 and S5-S6 for an overlay of different ^1H NMR spectra of the polymerizations). The evolution of the integral of the alkyne proton was followed over time in comparison to an internal standard (0.07 ppm from decamethylcyclotrisiloxane). The results of the consumption of HDDP over time are plotted in Figure 1.



Scheme 1 Polyadditions performed at the interface: (A) the system used for evaluating the kinetics of the polymerization between HDDP and BAP in an inverse miniemulsion (cyclohexane- d_{12} /D $_2$ O); (B) *in-situ* surfactant formation using the click reaction to stabilize a direct miniemulsion (water/styrene) during the radical polymerization of polystyrene nanoparticles. The different possible regioisomers 1,4- and 1,5- disubstituted 1,2,3- triazoles are highlighted here in red and blue, respectively.

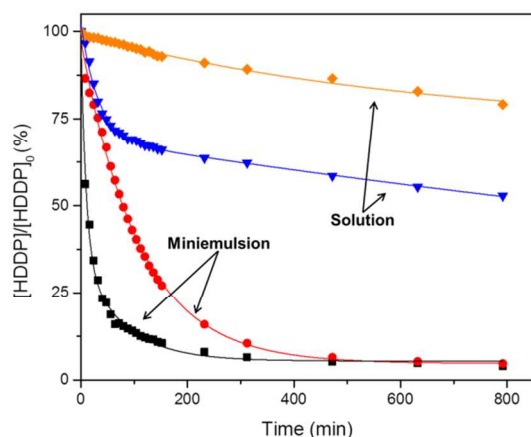


Fig. 1 Conversion of HDDP for the polyaddition with BAP in miniemulsion (water/cyclohexane $c=1.94 \times 10^{-2}$ mol/L) at 323 K (■) and 298 K (●) and in solution (DMSO- d_6 , $c=2.76 \times 10^{-2}$ mol/L) at 323 K (▼) and 298 K (◆), as determined by ^1H NMR.

As can be seen from Figure 1, the miniemulsion system resulted in a faster consumption of the HDDP monomer in comparison to solution polymerization. Considering the system as a second order reaction, the following method can be applied to obtain the values of k_2 :¹⁵

$$\frac{1}{1-x} = k_2 t, \text{ with } x = 1 - \frac{[\text{HDDP}]}{[\text{HDDP}]_0} \quad (1)$$

Applying this equation across the different reaction conditions for the first hour (Supporting Info. Fig. S7), at 298 K, the miniemulsion polymerization system showed a 25-fold increase in the value of k_2 compared to the solution polymerization. At 323 K a 12-fold increase was measured. The increase in the rate of reaction can be explained in terms of the so called “pseudo-phase model”, which relates the partitioning of monomers at the interface with the characteristics of the reagents with both solvents.^{16, 17} Bravo-Díaz and Romsted have modelled the reactivity of “macro”emulsions and “micro”emulsions in terms of the pseudo-phase model.¹⁷ Miniemulsions are kinetically stable systems, similar to macroemulsions, however with the additional kinetic stabilization against diffusion and collisions. The system studied herein uses a non-ionic surfactant, which avoids the interference of charges in the kinetics of the reaction, as observed by Engberts and coworkers for the kinetics of cycloadditions in microemulsions.¹⁸ Thus, this system is a better comparison to the solution polymerization. Two assumptions have been made for the application of the pseudo-phase model:

- the distribution of the components is a thermodynamic equilibrium, which can be represented by the partition coefficient;
 - the interfacial region is in a dynamic equilibrium state.¹⁷
- The partition coefficient depends on the solubility of the components in each of the solvents; therefore the level of acceleration observed will be directly linked to the chemical properties of the components of the system.

As an additional factor, two reaction temperatures were examined: for the miniemulsion polymerization, an increase on the k_2 -value from 298 K to 323 K of approximately 7-fold and for the solution polymerization an increase of approximately 15-fold was observed. This difference can be rationalized in terms of mobility. The increase of the temperature directly affects the Brownian mobility of the molecules in the system. In a miniemulsion system one monomer is confined inside the droplet and has limited space and therefore limited mobility at the interface, while in the homogeneous reaction both monomers have a similar mobility and are thus significantly more influenced by temperature variations.

The evolution of the molecular weight for both polymerization methods was followed by GPC (see Supporting Info Table S2 and Fig. S12). The data highlights that higher molecular weights are achieved in the miniemulsion systems at both temperatures compared to the solution polymerizations. This may be explained by considering two factors: (1) in solution the overall concentration is lower than at the interface of nanodroplets resulting in a decreased molecular weight of the polymer (up to $M_w=4500$ g/mol (vs. PS)); and (2) in the heterophase system, the polymer precipitates, and is confined to, the interface resulting in higher conversions and molecular weights (up to $M_w=10\,000$ g/mol in miniemulsion at 323 K). The final morphology obtained from the miniemulsion systems is, as expected, the formation of nanocapsules with a mean diameter of 190 ± 60 nm. This could be confirmed by SEM, TEM and light scattering measurements (see Supporting Info Fig. S13 and S14) and agrees well with what has been previously reported by our group.¹³ An interesting aspect of the interfacial reaction is whether there is an influence on the microstructure of the polymer. Tang and

coworkers¹⁰ have used metal-free alkyne-azide polymerization to produce a series of polymers with diazides and dipropiolates in solution and found a high tendency for the formation of 1,4-triazoles (over 90%). Both possible regioisomers (1,4- and 1,5-triazole) can be differentiated by two distinct singlets between 7 and 10 ppm in the ¹H NMR spectrum.¹⁹ Both products of the polymerization in solution and in miniemulsion were analysed via ¹H NMR spectroscopy (DMSO-*d*₆) and several signals can be detected in the triazole region (Fig. 2(A)). Interestingly, an inversion in the relative intensities of these peaks is observed depending on the polymerization methodology. Previously, ¹H-¹⁵N HMBC (hetero nuclear multiple bond correlation) NMR spectroscopy was used as a powerful tool to distinguish different triazole isomers.¹⁹ The ¹H - ¹⁵N HMBC analysis of the polymer obtained by miniemulsion polymerization is shown in Fig. 2(B). As can be clearly seen from the H-N correlation, the signals between 8.6 and 8.7 ppm (in the H NMR) are related to the same regioisomer, while the two signals around 8.24 ppm correlate to each other. The values are in agreement with the values observed by Alfonso and coworkers¹⁹ and indicated that the three signals at lower field of the spectrum stem from the 1,4-disubstituted 1,2,3-triazoles, while the two signals at higher field correspond to the 1,5-disubstituted 1,2,3-triazole derivatives. Miniemulsion thus preferentially produces the 1,4-triazole regioselective polymer (approximately 91%). Solution polymerization also predominates with the 1,4-isomer, however it appears to be of a different 1,4- species.

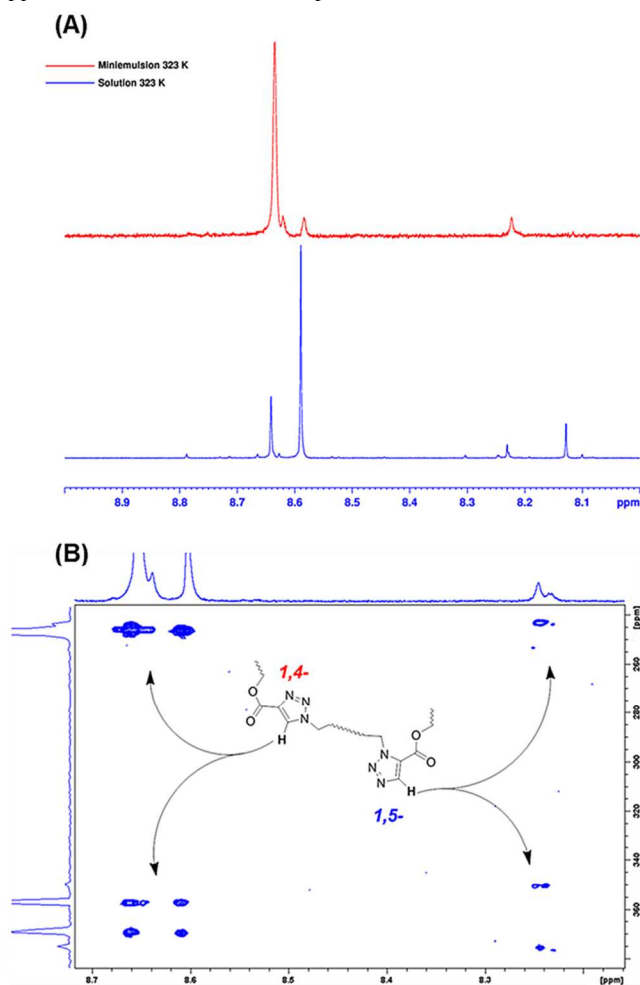


Fig. 2 (A) ¹H NMR spectra in DMSO-*d*₆ (700 MHz, Avance III) from the triazole proton region for the product of polymerization in

miniemulsion (red) and in solution (blue) at 323 K ; (B) 2D ¹H – ¹⁵N HMBC (850 MHz, Avance III) analysis of the polymer obtained by miniemulsion polymerization at 323 K in DMSO-*d*₆. The upper spectrum corresponds to the ¹H spectrum, the spectrum on the left is the internal calculation of the cross peaks in the ¹⁵N-frequency range.

To elucidate the nature of this second 1,4-derivative (8.65 ppm and 8.61 ppm), diffusion-ordered NMR spectroscopy (DOSY) ¹H-NMR analysis was performed. Fig. 3 shows the spectrum for the product of the miniemulsion polymerization at 323 K. The diffusion coefficient for the signal at ca. 8.61 ppm is higher (approximately $9.0 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$) than the one observed for the other peaks (approximately $5.5 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$). The fact that the peaks have a well-defined shape, suggests that it originates from a smaller, faster diffusing species of defined architecture and one that is not related to the molecular weight distribution of the polymer. A plausible explanation is the formation of cyclic oligomers. Considering the surface curvature of the droplet and the lower mobility of the reactants after precipitation at the interface, a lower probability for the formation of cyclic structures for the miniemulsion polymerization in comparison to the solution polymerization is expected. This assumption is in agreement with the change in the intensity between both methods observed in the 1D – ¹H NMR (Fig. 2(A)).

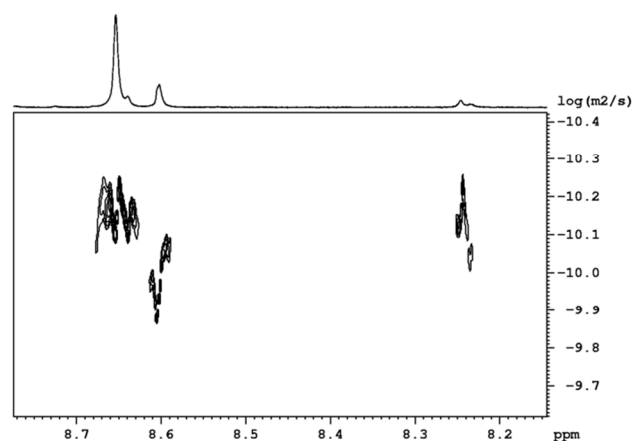


Fig. 3 DOSY ¹H – NMR (850 MHz, Avance III) measurement for the product of miniemulsion polymerization at 323 K in DMSO-*d*₆.

The miniemulsion polyaddition is a clear example of the interfacial “screw clamp” and has the potential to accelerate certain reactions, in mild conditions to give an easy pathway for the formation of amphiphilic molecules as protein conjugates or surfactants. To further exemplify this effect and the use of this approach in the preparation of amphiphilic molecules, a monofunctional system was used to generate a surfactant *in situ*, using mild conditions with the capability to stabilize a direct miniemulsion polymerization system. This is the first example for a chemical reaction that can be applied *in situ* to generate a surfactant that stabilizes a miniemulsion of monomer droplets for instantaneous polymerization. Previously, aliphatic carboxylic acids were deprotonated in heterophase to generate ionic surfactants^{20, 21} while metallosurfactants and transition metal acetyl acetonates have also been produced *in situ*.^{22, 23} The generation of a surfactant *in situ* can have advantages such as faster stabilization of the (mini)emulsion and less secondary nucleation processes.²⁴ Here, we used the interfacial “screw clamp” for mediating a click reaction in a direct miniemulsion between dodecyl

propiolate dispersed in styrene as the organic phase and *N*-ε-azido-L-lysine hydrochloride dissolved in the aqueous phase. The emulsion was produced by stirring and the subsequent application of ultrasound. The nanodroplets were formed with a high surface area and stabilized at the same time by the *in situ* generated, i.e. “clicked”, surfactant (compare Scheme 1 (B)). After the addition of a radical initiator (VA044) to the aqueous phase, a stable polystyrene nanoparticle dispersion with mean diameter of ca. 300 nm was obtained (Supporting Info Fig. S15).

Conclusions

Aqueous miniemulsion was used as a synthetic tool to mediate the metal-free 1,3-dipolar cycloaddition between propiolates and azides. A detailed kinetic investigation via NMR spectroscopy was conducted for the copper-free click polyaddition between HDDP and BAP. This $A_2 + B_2$ polyaddition was studied both in solution and in a biphasic system, i.e. miniemulsion, with one monomer being confined in the dispersed phase (water) and the second being dissolved in the continuous phase. Conducting the reaction in miniemulsion leads to a significant acceleration of the reaction leading to higher molecular weights at lower temperatures compared to the reaction in solution. Detailed NMR analyses of the microstructure of the final materials indicated that the polyaddition at the interface of miniemulsion droplets leads to the probable formation of less cyclic oligomers and a high regioselectivity towards the 1,4-triazole isomers.

The catalyst-free 1,3-dipolar cycloaddition in heterophase can be a powerful tool for bioapplications e.g. the encapsulation or bioconjugation of temperature sensitive biomolecules, such as proteins, that can be mediated at the water interface. Herein, we also used the interfacial “screw clamp” to generate a surfactant *in situ* as an example capable of stabilizing styrene nanodroplets of which could subsequently conduct a radical polymerization.

We believe that this approach is especially useful for biological materials due to bioorthogonality, mild conditions (low temperature, no catalyst) and the tolerance to water.

Notes and references

^a Max Planck Institute for Polymer Research, Ackermannweg 10, 55128, Mainz, Germany.

^b University of Applied Sciences Bingen, Berlinstrasse 109, 55411 Bingen, Germany.

* corresponding author: wurm@mpip-mainz.mpg.de

- S. Ding, G. Jia and J. Sun, *Angew. Chem. Int. Ed.*, 2014, **53**, 1877-1880.
- R. Huisgen, *Angew. Chem. Int. Ed.*, 1963, **2**, 633-645.
- V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2002, **41**, 2596-2599.
- C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057-3064.
- L. Zhang, X. Chen, P. Xue, H. H. Y. Sun, I. D. Williams, K. B. Sharpless, V. V. Fokin and G. Jia, *J. Am. Chem. Soc.*, 2005, **127**, 15998-15999.
- J. F. Lutz, *Angew. Chem. Int. Ed.*, 2008, **47**, 2182-2184.
- C. R. Becer, R. Hoogenboom and U. S. Schubert, *Angew. Chem. Int. Ed.*, 2009, **48**, 4900-4908.
- N. J. Agard, J. A. Prescher and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2004, **126**, 15046-15047.
- S. Sawoo, P. Dutta, A. Chakraborty, R. Mukhopadhyay, O. Bouloussa and A. Sarkar, *Chem. Commun.*, 2008, 5957-5959.
- H. Li, J. Wang, J. Z. Sun, R. Hu, A. Qin and B. Z. Tang, *Polym. Chem.*, 2012, **3**, 1075-1083.
- S. S. van Berkel, A. J. Dirks, S. A. Meeuwissen, D. L. L. Pingen, O. C. Boerman, P. Laverman, F. L. van Delft, J. J. L. M. Cornelissen and F. P. J. T. Rutjes, *ChemBioChem*, 2008, **9**, 1805-1815.
- K. Landfester, *Angew. Chem. Int. Ed.*, 2009, **48**, 4488-4507.
- J. M. Siebert, G. Baier, A. Musyanovych and K. Landfester, *Chem. Commun.*, 2012, **48**, 5470-5472.
- C. Tonhauser, A. Alkan, M. Schömer, C. Dingels, S. Ritz, V. Mailänder, H. Frey and F. R. Wurm, *Macromolecules*, 2013, **46**, 647-655.
- X. Liu, P. Du, L. Liu, Z. Zheng, X. Wang, T. Joncheray and Y. Zhang, *Polym. Bull.*, 2013, **70**, 2319-2335.
- Q. Gu, C. Bravo-Díaz and L. S. Romsted, *J. Colloid Interface Sci.*, 2013, **400**, 41-48.
- L. S. Romsted and C. Bravo-Díaz, *Curr. Opin. Colloid Interface Sci.*, 2013, **18**, 3-14.
- J. B. F. N. Engberts, E. Fernández, L. García-Río and J. R. Leis, *J. Org. Chem.*, 2006, **71**, 6118-6123.
- M. Corredor, J. Bujons, A. Messeguer and I. Alfonso, *Org. Biomol. Chem.*, 2013, **11**, 7318-7325.
- U. El-Jaby, M. Cunningham and T. F. L. McKenna, *Macromol. Chem. Phys.*, 2010, **211**, 1377-1386.
- G. Yi, L. T. Victoria, S. R. S. Ting and B. Z. Per, *Polym. J.*, 2012, **44**, 375-381.
- C. R. van den Brom, M. Wagner, V. Enkelmann, K. Landfester and C. K. Weiss, *Langmuir*, 2010, **26**, 15794-15801.
- C. R. van den Brom, N. Vogel, C. P. Hauser, S. Goerres, M. Wagner, K. Landfester and C. K. Weiss, *Langmuir*, 2011, **27**, 8044-8053.
- U. El-Jaby, M. Cunningham and T. F. L. McKenna, *Macromol. Rapid Commun.*, 2010, **31**, 558-562.

Table of contents (TOC)

Acceleration of a Huisgen alkyne-azide cycloaddition by the use of a miniemulsion approach was monitored by NMR techniques, demonstrating the potential of the interface to work as a “molecular screw-clamp”.

