

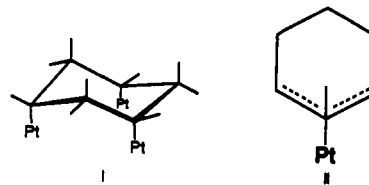
Figure 1 shows the results of a LITD/FTMS experiment in which cyclohexane was adsorbed at low coverage ( $\sim 0.03$  monolayer) on the Pt(111) surface at 150 K. This figure shows the intensity of the  $m/z$  56 peak ( $M - 28$ , the base peak in the electron ionization of cyclohexane) and the  $m/z$  78 peak (base peak for benzene) in the mass spectra which were obtained as the temperature of the Pt(111) sample was increased in a stepwise manner, as described above. At 150 K cyclohexane is stable on the surface and is the only species observed in the mass spectrum. As the Pt(111) crystal is heated to  $\sim 180$  K, the cyclohexane signal rapidly falls to 0, as shown in the figure. At this temperature, thermal desorption<sup>9</sup> and separate Auger electron spectra show that no thermal desorption is occurring, only surface dehydrogenation. At 180 K a small amount of benzene is observed, but it is not produced in substantial quantities until  $\sim 270$  K. Indeed, further experiments have led us to conclude that the benzene signal observed in the temperature range of  $\sim 180$ – $250$  K is due to a surface reaction of a tightly bonded intermediate caused by the laser-induced temperature jump.<sup>20</sup> While it may be coincidental, it should be noted that the onset temperature for benzene production on the surface ( $\sim 270$  K) corresponds closely to onset of recombination and desorption of the hydrogen atoms liberated in the reaction.

In the temperature range 180–270 K, the only signals observed in our low coverage LITD/FTMS spectra are due to a small amount of benzene which is formed by a laser-driven surface reaction. This suggests that the surface intermediate must be tightly bonded to the Pt(111) surface and is not desorbed in an intact manner by the laser-induced temperature jump. In an attempt to shed light on the nature of this surface intermediate, we have carried out experiments similar to the one described above but with initial adsorption of cyclohexene. In these experiments the starting temperature was reduced to 115 K. At this low surface temperature, cyclohexene is molecularly adsorbed and is easily observed in the LITD/FTMS spectra. At the low coverages used here, as the Pt surface temperature is increased, cyclohexene is stable up to  $\sim 150$  K, where the surface concentration decreases in a manner similar to that observed for cyclohexane. That is, the cyclohexene signal decreases but no new species appears until the surface temperature reaches  $\sim 270$  K, where the onset of benzene production is once again observed. While our previous work shows that cyclohexene is an intermediate in the dehydrogenation of cyclohexane, the present results show that cyclohexene is not the surface species that exists in the intermediate range of  $\sim 180$ – $270$  K, under the low-coverage conditions discussed here. We conclude, therefore, that cyclohexene reacts to form another surface species that is further dehydrogenated.

Additional LITD/FTMS experiments with low coverages of 1,3-cyclohexadiene and 1,4-cyclohexadiene showed that substantial amounts of benzene were observed in the mass spectra even after only 2 min at 115 K. This indicates that the activation energy for conversion of the cyclohexadienes to benzene is very low, and it clearly eliminates them as candidates for the surface species in the 180–270 K temperature range of the cyclohexane experiments.

We have now come to the conclusion that this surface intermediate is beyond cyclohexene ( $C_6H_{10}$ ) in the conversion to benzene but not yet dehydrogenated to the extent of the cyclohexadienes ( $C_6H_8$ ). These results suggest a stable surface intermediate of  $C_6H_9$  stoichiometry for the species that exists on the surface in the temperature range 180–270 K in the cyclohexane experiments and 150–270 K in the cyclohexene experiments. Two attractive possibilities for this intermediate are shown herein. Species I would result from the removal of the three axial hydrogens on the surface side of the chair form of cyclohexane. This has been suggested as an intermediate by vibrational spectroscopy studies of cyclohexane dehydrogenation on Pt<sup>5</sup> and investigated

by empirical electronic structure calculations.<sup>21</sup> Species II is an allylic  $C_6H_9$  species that could be easily formed from adsorbed cyclohexene by the loss of one  $\alpha$ -hydrogen.



While it seems likely from our experiments that the surface species that is stable in this intermediate range is of  $C_6H_9$  stoichiometry and may be I or II, we should stress that we have no direct evidence of the structure of this surface intermediate at this time and further studies by other surface spectroscopies are needed. Such studies will be greatly facilitated by the present results, which provide a well-defined temperature range and conditions for formation of the stable  $C_6H_9$  surface species. We have also shown here, for the first time, that benzene is not formed in substantial amounts until the surface is heated to  $\sim 270$  K, coincidental with the onset of recombinative desorption of the surface hydrogen produced in the reaction. These experiments also clearly demonstrate the potential of the LITD/FTMS technique for rapidly surveying the chemistry of complex adsorbate species.

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(21) Kang, D. B.; Anderson, A. B. *J. Am. Chem. Soc.* 1985, 107, 7858.

## Mutually Interpenetrating Inorganic–Organic Networks. New Routes into Nonshrinking Sol–Gel Composite Materials

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Control over bulk molecular architecture is a critical challenge in the design of new materials.<sup>1</sup> In the field of new inorganic–organic composites, aspects such as phase continuity, domain size, and molecular mixing at the phase boundaries greatly influence the optical, physical, and mechanical properties of the composite material.<sup>2</sup> Our interest in chemistry at the inorganic–organic interface has led to the design and preparation of new, homogeneous, optically transparent composite materials displaying a remarkably high degree of mixing between the two chemically dissimilar phases. These transparent composites are synthesized through a synchronous application of the aqueous ring-opening metathesis polymerization (aqueous ROMP)<sup>3</sup> of cyclic alkenyl

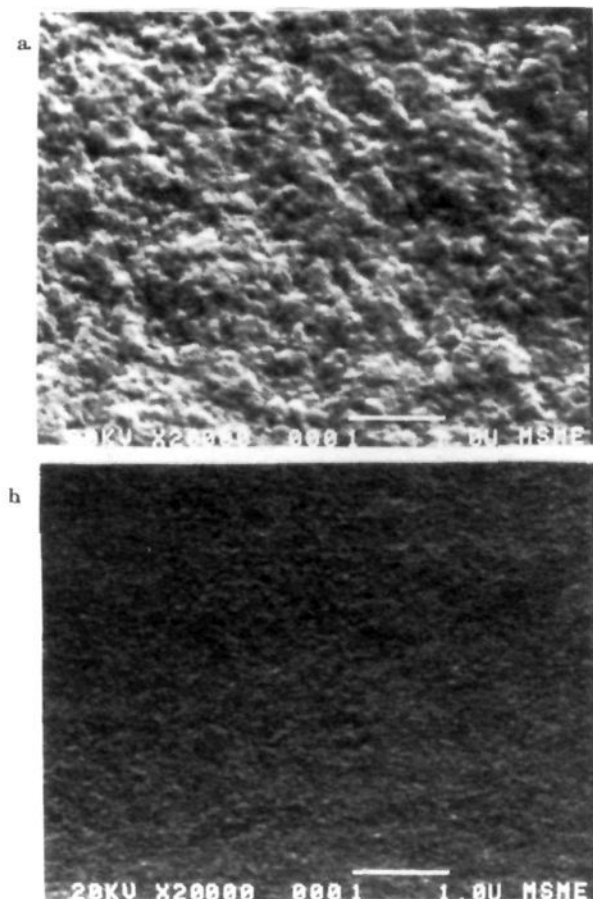
(19) Sherman, M. G.; Land, D. P.; Hemminger, J. C.; McIver, R. T., Jr. *Chem. Phys. Lett.* 1987, 137, 298.

(20) Parker, D.; Pettiette-Hall, C. L.; McIver, R. T., Jr.; Hemminger, J. C., unpublished results.

(1) (a) Klein, L. C., Ed. *Sol-Gel Technology for Thin Films, Fibers, Preforms, Electronics, and Specialty Shapes*; Noyes Publications: Park Ridge, NJ, 1988. (b) Manson, J. A.; Sperling, L. H. *Polymer Blends and Composites*; Plenum Press: New York, 1976.

(2) Sperling, L. H. *Interpenetrating Polymer Networks and Related Materials*; Plenum Press: New York, 1981.

(3) Novak, B. M.; Grubbs, R. H. *J. Am. Chem. Soc.* 1988, 110, 7542.

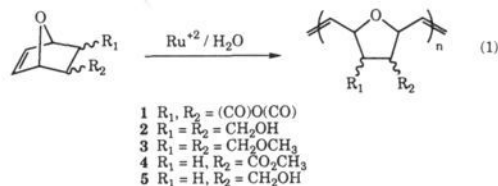


**Figure 1.** Comparison of SEM images (oxygen plasma etched) showing morphology and domain sizes of composites formed (a) by incorporating a preformed polymer (cellulose acetate, 30% by weight) into the inorganic network and (b) by synchronous formation of the organic polymer (poly(7-oxanorbornene-4,5-dicarboxylic acid), 30% by weight) and the inorganic network.

monomers and the hydrolysis and condensation of tetraalkyl orthosilicates (the sol-gel process).<sup>1,4,5</sup>

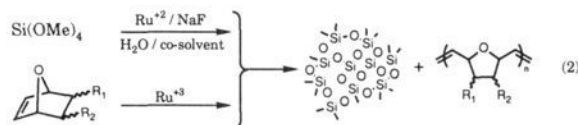
Sol-gel technology provides an attractive alternate route to the preparation of inorganic-organic composites.<sup>4</sup> The basic sol-gel process involves the sequential hydrolysis and polycondensation of metal alkoxides (usually tetraethyl orthosilicate (TEOS) or tetramethyl orthosilicate (TMOS)) in aqueous acid or base with a mutual cosolvent to form inorganic gels which can be dried to produce monolithic glasses.<sup>5</sup> The mild conditions offered by the sol-gel process allow for the incorporation of preformed organic polymers into the glass matrix to form composites possessing properties of both inorganic glasses and organic polymers.<sup>4</sup> However, due to the poor solvating properties of typical sol-gel formulations, homogeneous polymer solutions can only be obtained by using a limited number of polymers.<sup>4,6</sup> Compounding this

limitation, our experience has shown that several initially soluble polymers precipitate once gelation begins, resulting in uncontrolled phase separation and heterogeneous, opaque materials. Examples of these difficulties are provided by examining ROMP polymers. Their facile preparation,<sup>7</sup> high degree of functionality,<sup>8</sup> and extensive  $T_g$  range (ca. -100 to 250 °C)<sup>9</sup> make polymers derived from ROMP processes seemingly ideal candidates for use as composite polymers (eq 1).



Unfortunately, the preformed polymers were not compatible with the sol-gel solution. Poly-2 and poly-5 are completely insoluble in organic solvents. Poly-3 and poly-4 are soluble in organic solvents, but precipitate immediately upon addition of water to the solution. Poly-1 (hydrolyzed to the corresponding diacid under reaction conditions) remains soluble in the sol-gel solution, but phase-separates rapidly as gelation proceeds, resulting in a brittle, heterogeneous mixture possessing little mechanical integrity.

In an effort to circumvent the solubility and homogeneity problems associated with preformed polymers/sol-gel formations, we have investigated the formation of inorganic-organic simultaneous interpenetrating networks (SIPNs), wherein both inorganic glass and polymer formation occur concurrently (eq 2).



The aqueous  $\text{Ru}^{2+}$  ( $\text{Ru}(\text{H}_2\text{O})_6(\text{tos})_2$ ) and  $\text{Ru}^{3+}$  ( $\text{K}_2\text{RuCl}_5$ ) ROMP catalysts are stable to the sol-gel conditions (neutral to acidic aqueous solutions), and both ring-opening polymerization and glass formation proceed in almost quantitative yield.<sup>10</sup> Polymer to glass ratios as high as 1:2 have been obtained in most cases with no evidence of polymer precipitation or phase separation. It should be emphasized that *this simultaneous route allows for completely intractable and insoluble polymers (poly-2 and poly-5) to become homogeneously embedded within these sol-gel-derived glasses.*

Scanning electron microscopy (SEM)<sup>11</sup> studies indicate that the SIPN technique improves composite morphology by minimizing the phase separation between the inorganic glass and the

(4) (a) Wilkes, G. L.; Huang, H.; Carlson, J. G. *Polymer* **1989**, *30*, 2001. (b) Wilkes, G. L.; Orler, B.; Huang, H. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1985**, *26*, 300. (c) Huang, H.; Orler, B.; Wilkes, G. L. *Polym. Bull.* **1985**, *14*, 557. (d) Huang, H.; Orler, B.; Wilkes, G. L. *Macromolecules* **1987**, *20*, 1322. (e) Pope, E. J. A.; Asami, M.; MacKenzie, J. D. *J. Mater. Res.* **1989**, *4*, 1018. (f) Schmidt, H. *J. Non-Cryst. Solids* **1989**, *112*, 419. (g) Philipp, G.; Schmidt, H. *J. Non-Cryst. Solids* **1984**, *63*, 283. (h) Glaser, R. H.; Wilkes, G. L. *Polym. Bull.* **1988**, *19*, 51. (i) Huang, H.; Glaser, R. H.; Wilkes, G. L. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1987**, *28*, 434.

(5) (a) Ulrich, D. R. *CHEMTECH* **1988**, 242. (b) Mackenzie, J. D., Ulrich, D. R., Eds. *Ultrastructure Processing of Advanced Ceramics*; Wiley-Interscience: New York, 1988. (c) Ziegler, J. M., Fearson, F. W. G., Eds. *Silicon-Bases Polymer Science*; Advances in Chemistry 224; American Chemical Society: Washington, DC, 1990. (d) Wright, A. F., Dupuy, J., Eds. *Glass...Current Issues*; Martinus Nijhoff Publishers: Boston, 1985.

(6) (a) Wilkes, G. L.; Huang, H.; Glaser, R. H. In *Silicon Based Polymer Science*; Ziegler, J. M., Fearson, F. W. G., Eds.; Advances in Chemistry 224; American Chemical Society: Washington, DC, 1990. (b) Mark, J. E.; Ning, Y. D.; Jiang, C. Y.; Tang, M. Y.; Roth, W. C. *Polymer* **1985**, *26*, 2069.

(7) For recent references on metathesis, see: (a) Ivin, K. J. *Olefin Metathesis*; Academic Press: London, 1983. (b) Grubbs, R. H. In *Comprehensive Organometallic Chemistry*; Wilkinson, G., Ed.; Pergamon Press, Ltd.: Oxford, 1982; Vol. 8, pp 499-511. (c) Dragutan, V.; Balaban, A. T.; Dimonie, M. *Olefin Metathesis and Ring-Opening Polymerization of Cyclo-Olefins*, 2nd ed.; Wiley-Interscience: New York, 1985. (d) Novak, B. M.; Grubbs, R. H. In *Encyclopedia of Polymer Science and Engineering*, 2nd ed.; Mark, H. F., Bikales, N. M., Overberger, C. G., Menges, G., Kroschwitz, J. I., Eds.; John Wiley and Sons, Inc.: New York, 1990; Supplement Volume, 1990, p 420.

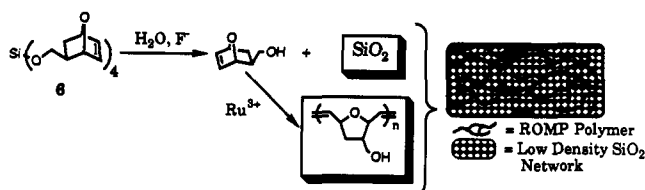
(8) (a) Novak, B. M.; Grubbs, R. H. *Polym. Mater. Sci. Eng.* **1987**, *57*, 651. (b) Novak, B. M.; Grubbs, R. H. *J. Am. Chem. Soc.* **1988**, *110*, 960.

(9) Novak, B. M. Ph.D. Thesis, California Institute of Technology, 1989.

(10) Representative SIPN synthesis: 100 mg of 3 and 5.0 mg of  $\text{K}_2\text{RuCl}_5$  are dissolved in 1.0 mL of ethanol and 1.0 mL of 47.5 mM NaF. The mixture is heated at 60° for 40 min. After 60 min, gelation is complete. The ruthenium is removed by immersion of the gel in 50% ethanol/dilute HCl for 48 h. A thin sheet of parafilm is placed over the mouth of the reaction flask, and the gel is slowly dried over 3 weeks. Verification of polymerization was accomplished by crushing the composite and extracting the polymer with  $\text{CHCl}_3$  (yield 96.4%).

(11) Scanning electron microscope images recorded on a JEOL-35C instrument at 20 kV, courtesy of John VanSlyke and Ronald Wilson, Department of Metallurgy and Mining, University of California, Berkeley, CA.

Scheme I



organic polymer. Contrast between the two phases can be best achieved by etching away (oxygen plasma)<sup>12</sup> the organic polymer to leave behind the textured glass surface, revealing cavities left by the polymer. Comparison of an etched SIPN composite with a transparent composite made from a preformed polymer (cellulose acetate) clearly displays a smaller domain size for SIPN composites over those materials derived from preformed polymers (Figure 1).

A significant drawback associated with the sol-gel process is removal of the cosolvent, excess water, and liberated alcohol. Xerogels routinely shrink 70–80% during the drying process,<sup>5,13</sup> which precludes molding processes and can introduce a high degree of stress within materials. Because of this shrinkage, gels must be dried at unreasonably slow rates to avoid cracking and deformation of the gel.<sup>5</sup> In an effort to overcome the problems associated with xerogel formation, we have synthesized tetraalkoxysilanes from SiCl<sub>4</sub> and the strained, cyclic alkenols 2 and 5.<sup>14</sup> By employing polymerizable monomers such as monomers 2, 3, 4, or 5 as the cosolvent, the tetraalkoxysilane derivative, and a stoichiometric quantity of water, all components of the composite solution contribute to either the SiO<sub>2</sub> network or the organic polymer. Since both the cosolvent and the liberated alcohol polymerize, gel drying is unnecessary and no gel shrinkage occurs (Scheme I).

Since the nonshrinking formulations are essentially bulk polymerizations, kinetic studies of the ROMP rates and condensation rates at high monomer concentrations were studied in order to verify simultaneous completion of both reactions. At high concentrations, the polymerization of alcohol 5 shows first-order behavior between 4.0 and 7.0 M ( $k = 1.9 \times 10^{-2} \text{ s}^{-1}$ ). At higher concentrations (pertinent for the bulk polymerization of 6), significant deviation toward faster rates is observed. Consistent with these observations, the ROMP of 5 is greater than 90% complete after 1.5 min. It is most convenient to match the sol-gel rates with these ROMP rates by adjusting the concentration of the condensation catalyst (NaF). For example, in the absence of ROMP catalysts, the condensation of compound 6 with stoichiometric water can be varied from several seconds to several days by simply adjusting the NaF concentration. To match the above ROMP rates, gel times of 1.5–2.0 min at 60 °C can be achieved by using NaF solutions near 50 mM. Significant deviations from these matched rates result in systems that approach the homopolymerization limits: uncontrolled polymer precipitation when the ROMP rate is greater than the condensation rate, or brittle glasses which shrink (due to unreacted monomer evaporation) when the condensation rate is much greater than the ROMP rate. Under ideal reaction conditions, a transparent glass-polymer composite is obtained with no observable shrinkage.

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(12) Etching performed with a Plasmod oxygen plasma etcher, courtesy of Dr. Donald Scales, California State Department of Public Health, Berkeley, CA.

(13) Brinker, C. J.; Scherer, G. W. *Sol-Gel Science. The Physics and Chemistry of Sol-Gel Processing*; Academic Press: New York, 1990.

(14) Ebelmen, J. J. *Justus Liebigs Ann. Chem.* 1846, 57, 331.

## Construction of a Light-Activated Protein by Unnatural Amino Acid Mutagenesis

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Low molecular weight caged substances, inactive precursor molecules that can be activated photochemically, are useful tools for biochemical studies.<sup>1–4</sup> Photolabile acyl enzyme adducts are also of interest in triggering enzymatic reactions.<sup>5</sup> We now report a general approach to the construction of *caged proteins* based on methodology that allows site-specific incorporation of unnatural amino acids into proteins.<sup>6,7</sup> This development should make possible a broad range of time-resolved experiments relevant to protein folding, protein-protein and protein-ligand interactions, catalytic mechanisms, and protein conformational studies. Here we describe the construction of a photoactivatable phage T4 lysozyme (T4L) containing an aspartyl β-nitrobenzyl ester (NB-Asp) in the active site.

T4L is a stable, well-characterized enzyme whose lysis of *Escherichia coli* cells provides a convenient and sensitive assay for catalytic activity.<sup>8</sup> Aspartic acid 20 (Asp 20) and glutamic acid 11 (Glu 11) oppose one another across the binding cleft floor and are essential for catalytic activity.<sup>8,9</sup> It has been proposed that Glu 11 of T4L donates a proton to the interglycosidic oxygen of β(1→4)-linked NAM-NAG residues of the cell wall and that the incipient oxonium ion decomposes to give a truncated polysaccharide and a carbocation which is stabilized by Asp 20.<sup>10</sup> An aspartyl β-nitrobenzyl ester was therefore introduced at position 20 to produce a catalytically inactive (caged) lysozyme. Upon irradiation, nitrobenzyl esters are known to undergo rapid (on the order of milliseconds<sup>11</sup>) conversion to the free acid plus *o*-nitrosobenzaldehyde. Irradiation of the inactive, NB-Asp-containing lysozyme thus generates a fully active protein.

Because suppression of the Asp 20 amber mutant is carried out *in vitro*, it was necessary to verify that protein synthesized *in vitro* is the same as protein synthesized *in vivo*. T4L<sup>12</sup> was expressed and purified to homogeneity from the cell-free coupled transcription-translation system of Zubay<sup>13</sup> programmed with the

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(1) (a) Schlichting, I.; et al. *Nature* 1990, 345, 309. (b) Schlichting, I.; et al. *Proc. Natl. Acad. Sci. U.S.A.* 1989, 86, 7687.

(2) (a) Blatt, M. R.; Thiel, G.; Trentham, D. R. *Nature* 1990, 346, 766. (b) Parker, I.; Ivorra, I. *Science* 1990, 250, 977. (c) Adams, S. R.; et al. *J. Am. Chem. Soc.* 1988, 110, 3212. (d) Adams, S. R.; Kao, J. P. Y.; Tsien, R. Y. *J. Am. Chem. Soc.* 1989, 111, 7957.

(3) Gilroy, S.; Read, N. D.; Trewavas, A. J. 1990, 346, 769.

(4) Wilcox, M.; et al. *J. Org. Chem.* 1990, 55, 1585.

(5) (a) Stoddard, B. L.; Bruhnke, J.; Porter, N.; Ringe, D.; Petsko, G. A. *Biochemistry* 1990, 29, 4871. (b) Stoddard, B. L.; Bruhnke, J. L.; Koenigs, P.; Porter, N.; Ringe, D.; Petsko, G. A. *Biochemistry* 1990, 29, 8042.

(6) (a) Noren, C. J.; et al. *Science* 1989, 244, 182. (b) Ellman, J. A.; et al. *Methods Enzymol.*, in press.

(7) Recently a similar approach has been used to incorporate unnatural amino acids into peptides; see: Bain, J. D.; et al. *J. Am. Chem. Soc.* 1989, 111, 8013.

(8) (a) Tsugita, A.; et al. *J. Biol. Chem.* 1968, 243, 391. (b) Tsugita, A. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: New York, 1971; Vol. 3, pp 343–411 and references therein.

(9) Weaver, L. H.; Matthews, B. W. *J. Mol. Biol.* 1987, 193, 189.

(10) Dao-Pin, S.; Liao, D.-I.; Remington, S. J. *Proc. Natl. Acad. Sci. U.S.A.* 1989, 86, 5361 and references therein.

(11) Pillai, V. N. R. *Synthesis* 1990, 1.

(12) The cysteine-free mutant T4L (C54T, C97A) was used in these studies because it is virtually identical with the wild-type protein in structure and activity, but does not suffer oxidative degradation; see: Perry, L. J.; Wetzel, R. *Biochemistry* 1986, 25, 733.

(13) (a) Zubay, G. *Annu. Rev. Genet.* 1973, 7, 267. (b) Collins, J. *Gene* 1979, 6, 29. (c) Pratt, J. In *Transcription and Translation: A Practical Approach*; Hames, B. D., Higgins, S. J., Eds.; IRL Press: Oxford, 1984; pp 179–209.