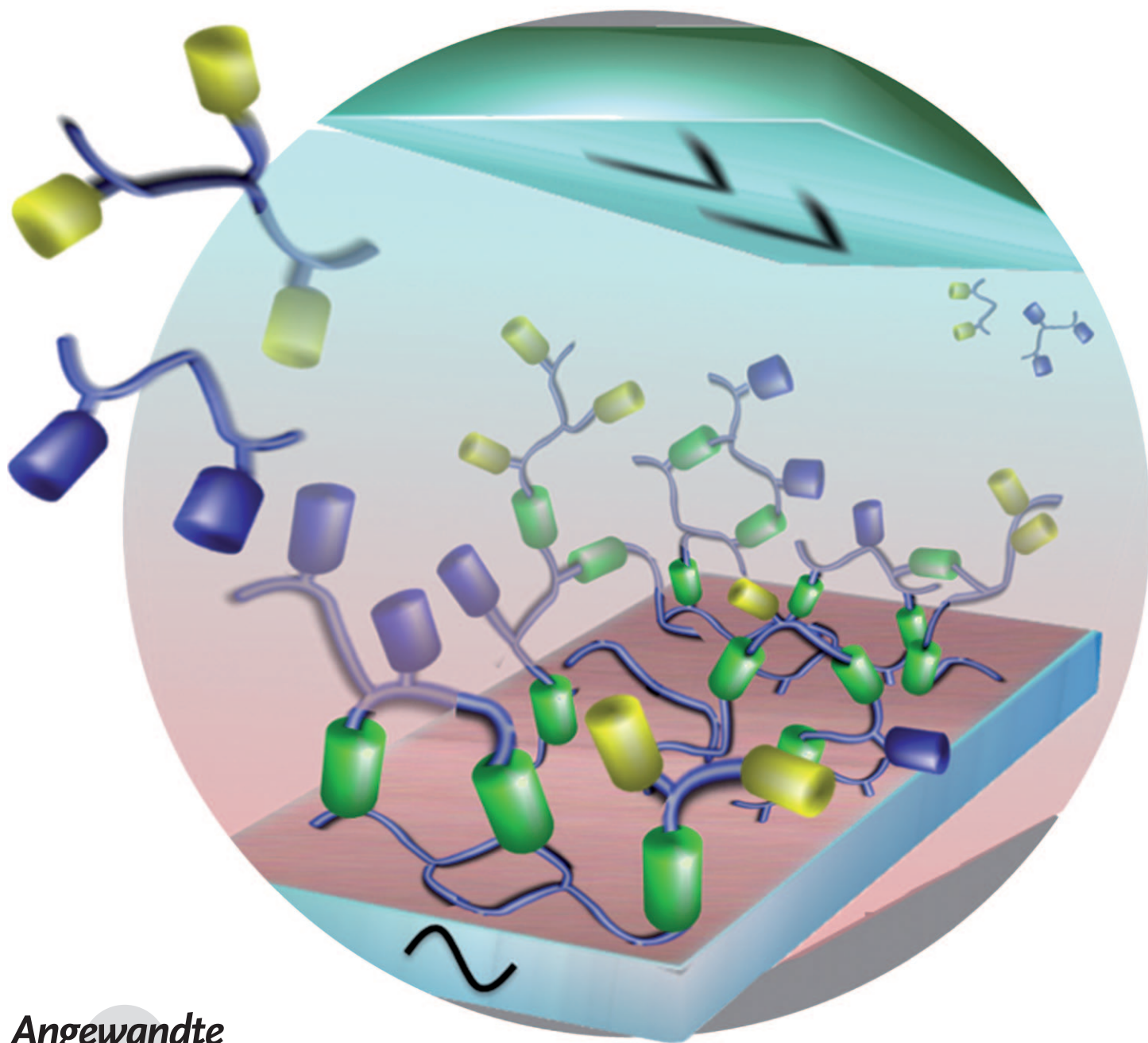# Electrochemically Triggered Assembly of Films: A One-Pot Morphogen-Driven Buildup**

Gaulthier Rydzek, Loïc Jierry, Audrey Parat, Jean-Sébastien Thomann, Jean-Claude Voegel, Bernard Senger, Joseph Hemmerlé, Arnaud Ponche, Benoît Frisch, Pierre Schaaf,* and Fouzia Boulmedais
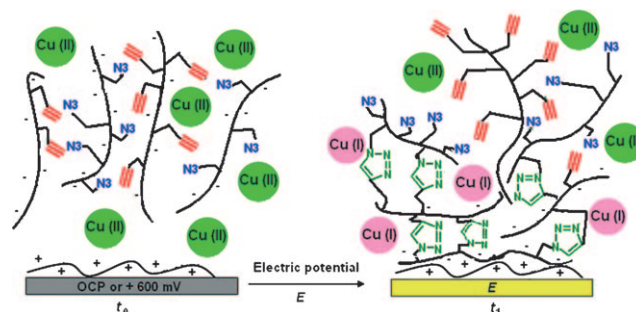
© 2011 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Angewandte Chemie

Self-assembly plays a central role in biology[1] and modern chemistry.[2] Except for self-assembled monolayers,[3] self-organized structures obtained by mixing interacting species are mainly observed in the bulk phase. Self-assembly taking place exclusively on surfaces and leading to films extending over the monolayer represents a real challenge. The main difficulty is the spontaneous and rapid interaction between the molecules as soon as they are mixed in solution. This can be circumvented by using different strategies, such as step-by-step deposition[4] or simultaneously spraying of the interacting species on the substrate.[5] In nature, biomineralization processes overcome the propensity of the different molecules to interact spontaneously by heterogeneous nucleation initiated by specific proteins.[6] At a higher structural level, formation of complex tissue morphologies is driven by morphogenetic fields.[7,8] These fields arise from the formation of gradients through production and diffusion of morphogens. According to Potter, morphogens are specific molecules to which cells respond in a concentration-dependent manner.[8] This definition can be extended to the formation of films on a substrate; the buildup of the film is triggered by the presence of molecules or ions, the morphogens, generated at or attracted by the substrate. A first example of morphogenetic self-assembly was recently introduced by Melosh and co-workers.[9] They described a dynamic polymerization of actin filaments from an electrode surface through ionic activation. This activation was obtained by diffusion of Mg^{2+} ions towards the electrode under the establishment of an electric double layer.[9]

Here, we introduce a new strategy to form films by generation of a morphogen at the substrate in the simultaneous presence of all the reactants in the solution. We illustrate our concept through the Cu^I-catalyzed click reaction.[10] We use two complementary functionalized polymers present simultaneously in the solution, one bearing azide groups and the other alkyne groups. Cu^I ions, which play the role of morphogens, are produced electrochemically in a continuous way from Cu^{II} ions present in the solution by application of a potential cycled between -350 and $+600$ mV (versus Ag/AgCl at a scan rate of 50 mV s^{-1}).[11] The Cu^I ions then diffuse from the surface towards the solution and catalyze the click reaction. This leads to the continuous buildup of a film through formation of triazole molecules between the azide- and alkyne-bearing units at the film/solution interface (Scheme 1).

[*] G. Rydzek, Dr. L. Jierry, Prof. P. Schaaf, Dr. F. Boulmedais
Institut Charles Sadron (UPR 22)
Centre National de la Recherche Scientifique
23 rue du Loess, 67034 Strasbourg (France)
Fax: (+33) 03-88-41-40-99
E-mail: pierre.schaaf@ics-cnrs.unistra.fr
Homepage: www.ics.u-strasbg.fr

Dr. A. Parat, Dr. J.-C. Voegel, Dr. B. Senger, Dr. J. Hemmerlé
Biomaterials and Tissue Engineering (UMR 977)
Institut National de la Santé et de la Recherche Médicale
11 rue Humann, 67085 Strasbourg (France)
and
Université de Strasbourg, Faculté de Chirurgie Dentaire
1 place de l'Hôpital, 67000 Strasbourg (France)

Dr. A. Ponche
Institut de Science des Matériaux de Mulhouse (LRC 7228)
Centre National de la Recherche Scientifique
15 rue Jean Starcky, BP 2488, 68057 Mulhouse (France)

Prof. P. Schaaf
Ecole Européenne de Chimie, Polymères et Matériaux
Université de Strasbourg
25 rue Becquerel, 67087 Strasbourg (France)
and
International Center for Frontier Research in Chemistry
8 allée Gaspard Monge, 67083 Strasbourg (France)
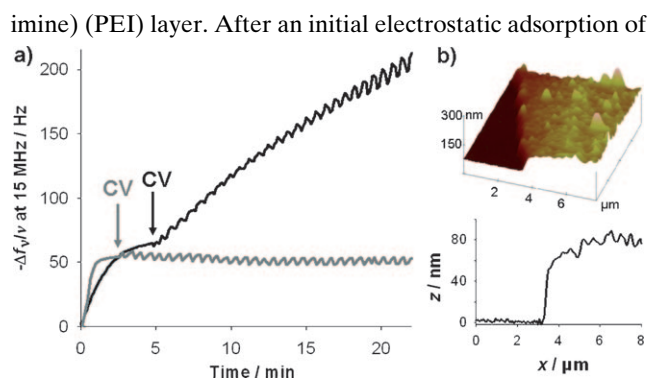
Dr. J.-S. Thomann, Dr. B. Frisch
Laboratoire de Conception et Application de Molécules Bioactives
UMR 7199, CNRS/Université de Strasbourg, 74 route du Rhin
67400 Illkirch (France)

Scheme 1. One-pot morphogen-driven formation of films using electrochemically controlled click chemistry.

The validity of the concept was tested on several systems based on functionalized polyanions (poly(acrylic acid), PAA), polycations (poly(allylamine hydrochloride), PAH), and neutral polymers (poly(*N*-hydroxypropylmethacrylamide), PHPMA). These polymers were modified either with alkyne (PAA$_{C≡C}$, PAH$_{C≡C}$, PHPMA$_{C≡C}$) or with azide (PAA$_{N3}$, PAH$_{N3}$, PHPMA$_{N3}$) functions grafted at 5% through ethylene oxide (EO) arms. A bifunctional alkyne spacer $HC≡C-(CH_2-CH_2O)_3-C≡CH$ ($_{C≡C}$-EO$_{3-C≡C}$) was also used. The films formed from the following solutions were investigated: PAA$_{C≡C}$–PAA$_{N3}$, PHPMA$_{C≡C}$–PHPMA$_{N3}$, PAH$_{C≡C}$–PAH$_{N3}$, PAA$_{C≡C}$–PHPMA$_{N3}$, PHPMA$_{C≡C}$–PAA$_{N3}$, and $_{C≡C}$-EO$_{3-C≡C}$–PAA$_{N3}$; the concentration of all polymers was 0.5 mg mL^{-1}. The buildups were monitored by an electrochemical quartz crystal microbalance (EC-QCM) which we used for both applying a controlled potential on the gold electrode and measuring the film thickness. The buildup of the film was performed under a slight flux (0.1 mL min^{-1}) to ensure a constant concentration of reactants. The polymer formulae and synthesis are given in the Supporting Information together with the experimental conditions for the buildup.

Figure 1a shows a typical evolution of the signal for the PAA$_{C≡C}$–PAA$_{N3}$ system. To strongly anchor the film on the surface, the substrate was precoated with a poly(ethylene

imine) (PEI) layer. After an initial electrostatic adsorption of

Figure 1. a) Evolution of the normalized frequency shift, measured at 15 MHz ($\nu=3$) by EC-QCM, of a $PAA_{C\equiv C}$–PAA_{N3} mixture (black line) and nonfunctionalized PAA (gray line) 0.6 mM $CuSO_4$ solution during the application of a potential cycled between -350 and $+600$ mV (versus Ag/AgCl, scan rate of 50 mVs^{-1}). After the electrostatic adsorption of polymers on a PEI-precoated surface, CV was applied as indicated by the black and gray arrows. b) Typical AFM 3D image (8 μm×8 μm; contact mode) of a scratched $PAA_{C\equiv C}$–PAA_{N3} film, obtained after 120 min, observed in the liquid state at pH 3.5 and its cross-section profile.

the PAA polymers (5 minutes), the application of cyclic voltammetry (CV) induced a continuous increase in the absolute value of the normalized frequency shift ($-\Delta f_v/\nu$). Small oscillations are superimposed on this continuous increase as a result of the cyclic electrochemical formation and destruction of a Cu^0 layer.[11] When nonfunctionalized PAA is used in the presence of Cu^{II} ions in solution, only the small oscillations persist when CV is applied (Figure 1a). Only the initial electrostatic adsorption of PAA polymers on PEI is observed in the absence of Cu^{II} ions (Figure S1 in the Supporting Information). This proves that the continuous signal increase in the simultaneous presence of functionalized PAA and Cu^{II} ions is due to a continuous film buildup. Qualitatively similar evolutions were observed for the other investigated systems (see Figures S2 to S4 in the Supporting Information). To remove any trace of Cu ions, we brought the film in contact with a 0.02 M EDTA solution at pH 3.5. After this treatment, CV (in 2.6 mM $NaNO_3$ solution) showed the disappearance of the Cu^{II} signal without any change in the film thickness (Figure S5 in the Supporting Information). The absence of copper traces was confirmed by X-ray photoelectron spectroscopy (XPS) analysis (Figure S6 in the Supporting Information).

XPS measurements confirmed the formation of triazole units resulting from the reaction between azide and alkyne groups. Indeed two peaks were observed, one centered at 401.9 eV characteristic of the N 1s orbital of the central nitrogen of the triazole and one centered at 400.1 eV corresponding to both N 1s orbitals of the two lateral nitrogen atoms of the triazole units and to the nitrogen atoms of the amide bonds from functionalized PAA (Figure S7 in the Supporting Information). The ratio between the areas of these two peaks is in agreement with an almost complete reaction between the azide and alkyne groups (see detailed discussion in the Supporting Information). In addition, the

peaks centered at 401 and 405 eV characteristic of azides[12] are absent from the XPS spectrum. These results provide strong evidence of a quasiquantitative reaction between the two functionalized PAA polyanions.

The film morphology was also investigated by atomic force microscopy (AFM). Figure 1b displays a typical 3D image and the cross-section profile of a scratched $PAA_{C\equiv C}$–PAA_{N3} film obtained by application of CV over 120 minutes. Typical topography images of $PAA_{C\equiv C}$–PAA_{N3} films are given in Figure S8 in the Supporting Information. The deposited organic $PAA_{C\equiv C}$–PAA_{N3} layer covers the surface totally with a thickness of 83 nm at pH 3.5 and is rather smooth as shown by the surface roughness (7 nm). When this film was put into contact with an aqueous solution at pH 9 where PAA (pK_a 4.5) is fully charged,[13] the film thickness increased to 355 nm but the surface topography and roughness remained largely unchanged (Figures S8 and S9 in the Supporting Information). A totally reversible swelling/deswelling process was observed by switching the pH back and forth between 3.5 and 9, proving the film robustness and stability.

We also investigated the effect of the Cu^I concentration which was varied in two ways: by changing the Cu^{II} concentration in the solution (Figure 2) and by varying the lowest limit of the applied potential range (Figure S10 in the Supporting Information). In both cases, reducing the production of Cu^I at the electrode results in thinner films. One can observe a reduction of the thickness increase as the buildup progresses. This is not due to a decrease of the Cu^I generation during the buildup process (Figure S11 in the Supporting Information) but might be due to a limited diffusion of Cu^I towards the film/solution interface.[11]
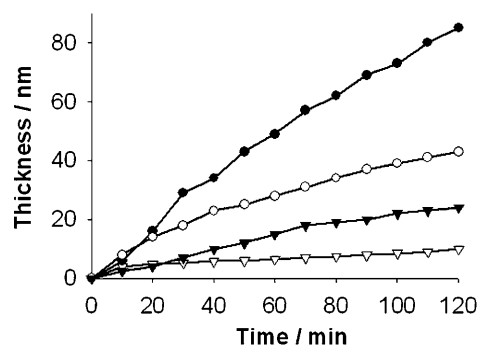
Figure 2. Evolution of the thickness of the $PAA_{C\equiv C}$–PAA_{N3} films, built on PEI-precoated surfaces, obtained in the presence of 0.6 (●), 0.45 (○), 0.3 (▼), and 0.075 mM (▽) solutions of $CuSO_4$ as a function of the applied time of the potential cycling between -350 and $+600$ mV (versus Ag/AgCl, scan rate of 50 mVs^{-1}). The film thickness was calculated from EC-QCM data (see the Supporting Information), by subtracting the contribution of the electrostatic adsorption on PEI.

Film formation can also take place through passive morphogen production. First we deposited a layer of Cu^0 on the electrode from a $CuSO_4$ solution by applying a potential of -350 mV. After switching off the potential and bringing the Cu^0 layer in contact with a $PAA_{C\equiv C}$–PAA_{N3} solution in the presence of a 0.6 mM $CuSO_4$ solution, we observed a fast film

buildup followed by a leveling off (Figure S12 in the Supporting Information). In contrary, the direct presence of stabilized Cu^I ions in the polymer solution, that is, tetrakisacetonitrile Cu^I triflate, instead of electrochemically generated Cu^I ions, did not lead to a film buildup on the substrate (Figure S13 in the Supporting Information). The localized generation of morphogens, that is, Cu^I ions, in the vicinity of the surface is thus necessary for the film formation and its concentration has an influence on the thickness of the obtained film.

This new concept of film buildup is not restricted to copper ions and the click reaction but can also be applied to supramolecular coordination-driven assemblies (Figure S14 in the Supporting Information). A one-pot morphogen-driven assembly based on PAA-modified terpyridine ligands was built by coordination complexes with Ru^{II} or Fe^{II} ions (Figures S15 and S16 in the Supporting Information). These morphogen ions were produced electrochemically from surfaces where Ru^0 or Fe^0 was deposited before starting the buildup of the film. This approach is conceptually different from the electropolymerization process. Electropolymerization is restricted to monomer incorporation and is based on polymerization at the electrode that propagates through polymeric chains. It is also different from layer-by-layer multilayer films which are made from repetitive step-by-step processes.

In its present stage, the concept seems to be restricted to systems where the morphogen can be produced electrochemically at an interface. Yet, other methods to produce morphogens might eventually arise, such as the buildup at a semipermeable membrane where the morphogen could diffuse through; these approaches are to be developed.

[1] S. Camazine, J.-L. Deneubourg, N. R. Franks, J. Sneyd, G. Theraulaz, E. Bonabeau, *Self-Organization in Biological Systems*, Princeton University Press, Princeton, **2001**.

[2] G. M. Whitesides, M. Boncheva, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4769.

[3] A. Ulman, *Chem. Rev.* **1996**, *96* 1533–1554.

[4] G. Decher, *Science* **1997**, *277*, 1232–1237.

[5] C. H. Porcel, A. Izquierdo, V. Ball, G. Decher, J.-C. Voegel, P. Schaaf, *Langmuir* **2005**, *21*, 800–802.

[6] G. K. Hunter, H. A. Goldberg, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 8562–8565.

[7] S. E. Millar, *J. Invest. Dermatol.* **2002**, *118*, 216–225.

[8] J. D. Potter, *Nat. Rev. Cancer* **2007**, *7*, 464–474.

[9] I. Y. Wong, M. J. Footer, N. A. Melosh, *J. Am. Chem. Soc.* **2008**, *130*, 7908–7915.

[10] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056–2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.

[11] G. Rydzek, J. S. Thomann, N. B. Ameur, L. Jierry, P. Mésini, A. Ponche, C. Contal, A. E. El Haitami, J.-C. Voegel, B. Senger, P. Schaaf, B. Frisch, F. Boulmedais, *Langmuir* **2010**, *26*, 2816–2824.

[12] Y. C. Tang, G. M. Liu, C. Q. Yu, X. L. Wei, G. Z. Zhang, *Langmuir* **2008**, *24*, 8929–8933.

[13] D. Yoo, S. S. Shiratori, M. F. Rubner, *Macromolecules* **1998**, *31*, 4309–4318.