Synergistic Effect of Electric Field and Ultrasound on Transdermal Transport

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Received August 9, 1995; accepted January 20, 1996

KEY WORDS: transdermal drug delivery; electroporation; sonophoresis; ultrasound; stratum corneum.

INTRODUCTION

Transdermal drug delivery offers a substitute method to oral drug delivery and injections for drug administration (1). However, its applications are currently limited to only a few drugs due to the low permeability of the stratum corneum (SC), the outermost layer of the skin. The SC consists of flat dead cells (keratinocytes) surrounded by lipid bilayers, whose highly ordered structure confers an impermeable nature to the SC. Various approaches including chemical enhancers (2), ultrasound (3–4) and electrical enhancement (5–8) have been suggested to enhance transdermal drug transport. In some cases, high strengths of the physico-chemical forces (for example, electricity, ultrasound) are required to deliver a given drug dose transdermally. However, the highest strength of these physicochemical forces that can be used is likely to be limited by their adverse physiological effects. It is possible that simultaneous application of more than one physico-chemical force may decrease the required strength of these forces to achieve a given transdermal transport enhancement.

Electroporation involves application of electric field pulses that create transient aqueous pathways in lipid bilayer membranes, causing a temporary alteration of skin structure. While occurrence of aqueous pores may allow transdermal permeation of neutral molecules by diffusion, the transport of charged molecules during pulsing occurs predominantly by electrophoresis and electrosmosis (5–7). On the other hand, application of ultrasound has been suggested to enhance skin permeability due to cavitation which induces disorganization of the stratum corneum lipid bilayers (4). In addition, occurrence of convective transport during sonophoresis has also been suggested (3). Because the mechanism by which ultrasound and electric field enhance transdermal transport are different, we hypothesized that their combination might result in a synergistic effect.

In this note, we examine whether the transdermal transport enhancement induced by simultaneous application of ultrasound and electric pulses is higher than that due to electric pulses or ultrasound alone in the case of two model compounds, calcein and sulphorhodamine. We show that application of ultrasound reduces the threshold voltage required for the onset of calcein and sulphorhodamine transport in the presence of electric fields. We also suggest possible mechanisms for the synergistic effect of ultrasound and electric field on transdermal transport.

MATERIAL AND METHODS

Materials

Full thickness human cadaver skin (obtained from local hospitals) was heat stripped by immersion in 60°C water for two minutes followed by the removal of the epidermis. The skin was then stored in a humidified chamber (95% relative humidity) at 4°C. The heat stripped human epidermis was placed in a custom-made side-by-side permeation chamber, skin area of 0.64 cm^2, designed to adapt an ultrasound transducer at the donor side (Figure 1). The donor compartment was filled with a 1mM solution of calcein (MW 623, electric charge −4; Sigma Chemicals) and 1mM sulphorhodamine (MW 607, electric charge −1; Sigma Chemical) in 150 mM Phosphate Buffer Saline (PBS; Sigma Chemicals). Fresh PBS was continuously pumped into the receptor compartment at 0.8 ml/min. from a reservoir. The effluent from the receptor compartment was pumped through a spectrofluorometer (Fluorolog-II-system F112AI SPEX-industries, Edison, NJ) where the fluorescence of calcein and sulphorhodamine was separately measured twice every minute.

Fluorescence Measurements

The fluoremeter was set up for dual wavelength measurements (excitation wavelength = 488 nm, emission wavelength = 515 nm (calcein), and excitation wavelength = 586 nm, emission wavelength = 607 nm (sulphorhodamine)). The sample cuvette of the fluoremeter was sealed but for two openings that were provided for the flow of receiver fluid through it. A small custom-made electric stirrer was installed in the cuvette so that there were no stagnant zones in it. Care was taken to avoid any obstruction of the excitation beam by the stirrer. The time resolution of the flow-through fluorescence measurement system and subsequent calculations are described elsewhere (6). Transdermal calcein and sulphorhodamine flux was calculated from the fluorescence readings by taking into account parameters such as flow rate, receiver compartment volume, and fluoremeter caveat volume.

Application of Ultrasound

Ultrasound was applied under therapeutically approved conditions (1.4W/cm^2, 1MHz and 3 MHz, continuous) using a sonicator (Sonopuls 463, Henley International) for various exposure times up to 1 hour. The ultrasound transducer was located at a distance of about 3 cm from the skin.

Electroporation

One Ag/AgCl electrode (In vivo metric, Healdsburg, CA) was located in the donor and one in the receptor compartment, so that the distance of electrodes from the skin was equal in

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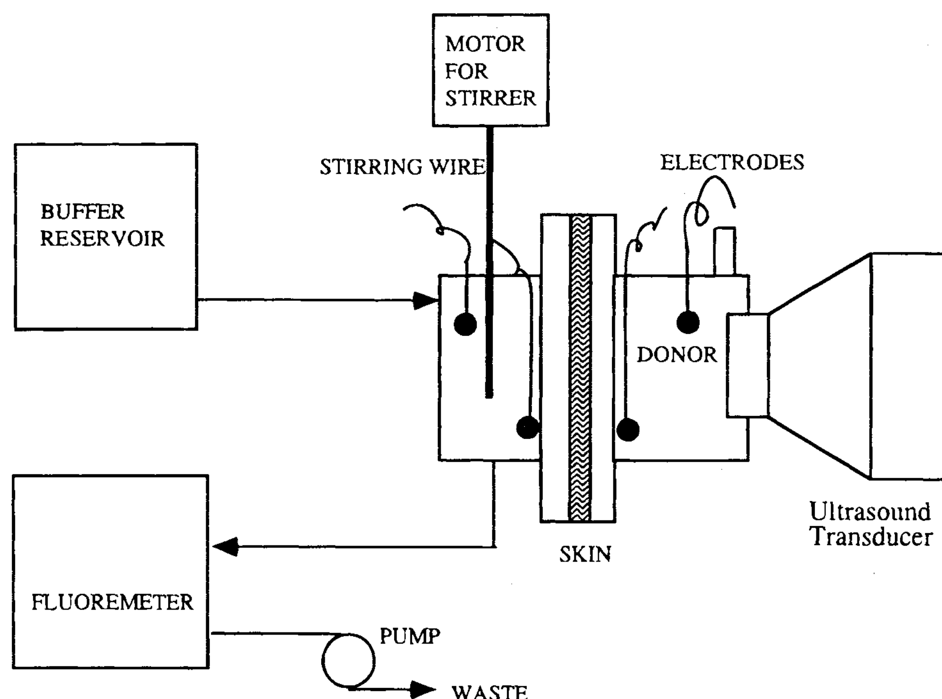

Fig. 1. Experimental set-up for simultaneous application of ultrasound and electric voltages.

both the compartments (about 8 mm). Voltage pulses were applied using a pulse generator (ECM 600, BTX, San Diego, CA) across these electrodes such that the positive electrode was always in the receptor compartment. This provided an electric driving force for calcein and sulphorhodamine (both negatively charged) to transport across the skin. The voltage applied to the electrodes divides between the saline and the skin. The voltage drop across the skin was estimated using the measured electrical resistance of the skin and saline. The magnitude as well as the length of the voltage pulses was varied over a wide range in order to investigate their effect on transdermal transport. The specific protocols concerning electroporation are presented in Results and Discussion.

In order to assess the stability of these molecules during electroporation, calcein and sulphorhodamine solutions (1 mM each) were exposed to electroporating conditions similar to those used in this study. No difference between the intensity of their fluorescence before and after exposure to electric fields could be detected. In addition, we found that these molecules are stable up to a temperature of 100°C (measured in terms of fluorescence). We also found that when these molecules are degraded, they do not fluoresce. In general, these molecules have been found to be very stable against many physico-chemical changes (9, 10).

Measurements of Passive Electric Skin Properties

A second pair of electrodes (same type as above) was used for monitoring the passive electrical properties (specifically, electric resistance) (11). Since the electric resistance of the skin is a good indicator of its barrier properties, we measured the skin resistance before, during and after our experiments. The effect of electroporation and ultrasound separately and together on skin electric resistance was determined. If the electrical resistivity before the application of either ultrasound or electroporation was lower than 20kΩ-cm^2 or if any significant passive calcein or sulphorhodamine transdermal flux was observed (that is, $J > 0.002 \mu g/cm^2/h$ (our detection limit)) the skin piece was considered leaky and replaced by a new piece.

RESULTS AND DISCUSSION

Application of Ultrasound Enhances the Efficacy of Electric Field

Figure 2A and 2B show the effect of simultaneous application of ultrasound (1 MHz, 1.4 W/cm^2, continuous application) and electric field (100 V across the skin, exponentially decaying pulse with a time constant (τ) of 1 millisecond, one pulse applied every minute) on the transdermal transport of calcein and sulphorhodamine respectively. The passive transdermal transport (in the absence of ultrasound and electric field) is below our detection limit and is not shown in Figure 2A or 2B. Application of ultrasound alone does not enhance the flux of calcein or sulphorhodamine. However, application of ultrasound enhanced steady-state transdermal flux of both, calcein and sulphorhodamine during elecrtic field pulsing. The enhancement is quantitatively defined as the amount of calcein or sulphorhodamine transported in the presence of ultrasound-electric field pulsing to that in the presence of electric field pulsing alone. This ratio is 2 in the case of calcein, and 3 in the case of sulphorhodamine (Figure 2A and 2B). Application of ultrasound also reduces transdermal calcein transport lag time, defined as the time required to reach the steady state, from a typical value of 15 minutes in the presence of electric field alone to about 9 minutes in the presence of ultrasound and electric field.

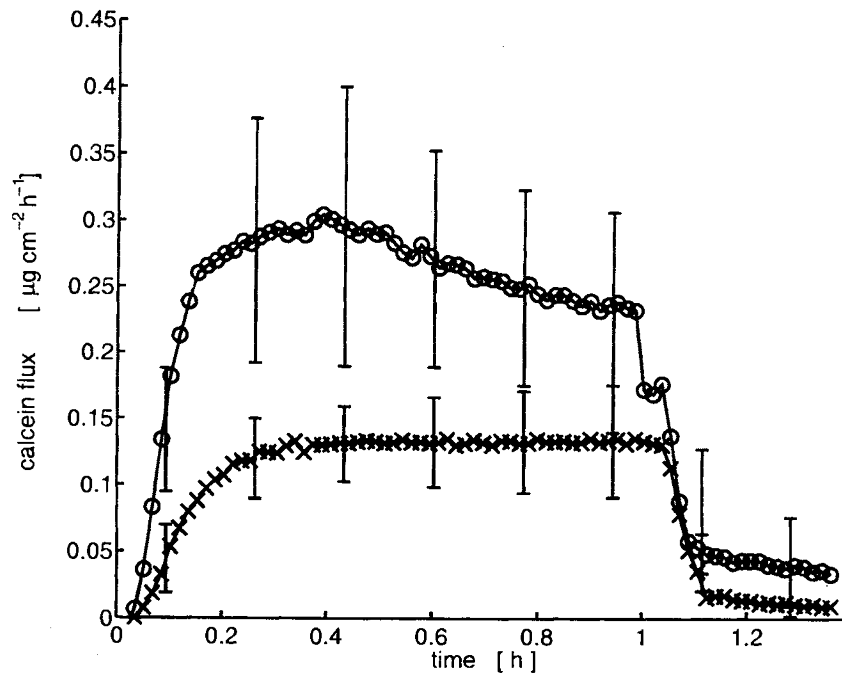

Fig. 2A. Time variation of calcein flux in the presence of electric fields alone (X) and during simultaneous application of ultrasound and electric field (O). Ultrasound was ON all the time (O). Electric voltage was turned ON at time 0 and was turned OFF at 1 hour in both the case (O as well as X). Presented as means and S.D. of at least three repetitions.

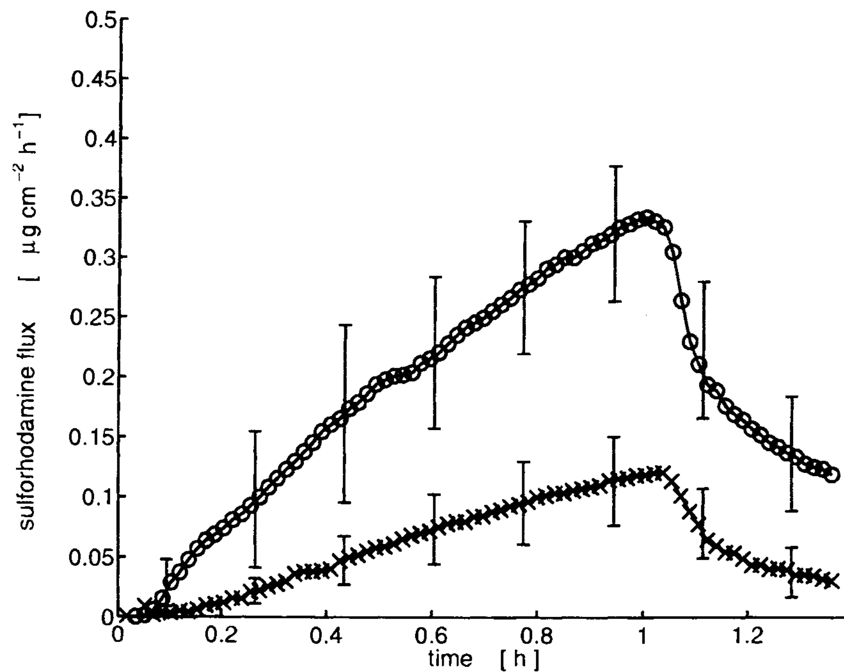

Fig. 2B. Time variation of sulphorhodamine flux in the presence of electric field alone (X) and during simultaneous application of ultrasound and electric field (O). Ultrasound was ON all the time (O). Electric voltage was turned ON at time 0 and was turned OFF at 1 hour in both the case (O as well as X). Presented as means and S.D. of at least three repetitions.

In order to quantitatively estimate the reduction in the required pulsing voltages by simultaneous application of ultrasound, we measured transdermal sulphorhodamine transport in the presence as well as absence of ultrasound (1 MHz, 1.4 W/cm^2) and electric field (voltage across the skin increased from 20 V to 150 V in steps of 5V every 30 minutes, 1 millisecond exponential pulse applied every minute). Figure 3 shows the variation of transdermal sulphorhodamine flux with voltage across the skin in the presence (O) as well as in the absence (X) of ultrasound. The transdermal sulphorhodamine flux is nearly zero as long as the voltage is below the threshold value and thereafter increases linearly with voltage. The threshold voltage for this pulsing protocol can be estimated by measuring the intercept of the linear variation of flux with voltage on the voltage axis. In the absence of ultrasound, this threshold is about 53 $\pm$ 3 V and that in the presence of ultrasound is about 46 $\pm$ 3 V (see Figure 3) indicating that application of ultrasound slightly reduces the threshold pulsing voltage. Figure 3 also shows that the transdermal sulphorhodamine flux at various pulsing voltages is always higher in the presence of ultrasound. Thus, the pulsing voltage required to achieve a given transdermal flux is smaller in the presence of ultrasound. For example, to achieve a transdermal sulphorhodamine flux of 0.15 μg/cm^2/hr, the required voltage is about 95 V in the absence of ultrasound and 75 V in the presence of ultrasound.

In order to assess whether application of ultrasound induces any irreversible change in the skin structure, we performed the following experiment. We first exposed human skin pieces to electric field alone (100 V across the skin, exponentially decaying pulse with a time constant (τ) of 1 millisecond, one pulse applied every minute), then simultaneously to ultrasound (1 MHz, 1.4 W/cm^2)-electric field and again to electric field alone. A comparison of sulphorhodamine transport due to the electric field alone, before and after the simultaneous electric field-ultrasound treatment, indicated that the flux returns to a near baseline value (data not shown), suggesting that the application of ultrasound did not induce any irreversible alteration in the barrier properties of skin. The recovery was also supported by electric resistance measurements indicating that application of ultrasound did not cause any irreversible change in the electrical resistance of the skin (data not shown).

Possible Mechanisms for the Synergistic Effect of Ultrasound and Electric Field

It has been recently shown that the application of ultrasound induces cavitation in the keratinocytes of the stratum corneum (4). Furthermore, oscillations of cavitation bubbles were shown to induce a partial disorder in the skin lipid bilayer (4). In view of this, we hypothesize that the synergistic effect of ultrasound and electric field may also be related to cavitation induced by ultrasound exposure. In order to test this hypothesis, we simultaneously exposed the skin to electric pulses (100 V across the skin, 1 ms exponential pulse applied every minute) and ultrasound (*3 MHz,* 1.5 W/cm^2). It is known that the cavitational effects vary inversely with ultrasound frequency (12). No significant cavitational effects have been observed in fluids at high ultrasound frequencies > 2.5 MHz (11). As a result, 2.5 MHz is considered a reasonable estimate of the upper frequency limit for the occurrence of cavitation in fluids at therapeutic ultrasound intensities. Hence, if cavitation plays an important role, the synergistic effect of ultrasound and electric field should be nearly absent when 3 MHz ultrasound is used. Indeed, we found that exposure to ultrasound at 3 MHz (intensity = 1.5 W/cm^2) does not affect transdermal transport by electric field pulsing (data not shown). These results indicate that cavitation may play a major role in the synergistic effect of ultrasound and electric field pulsing.

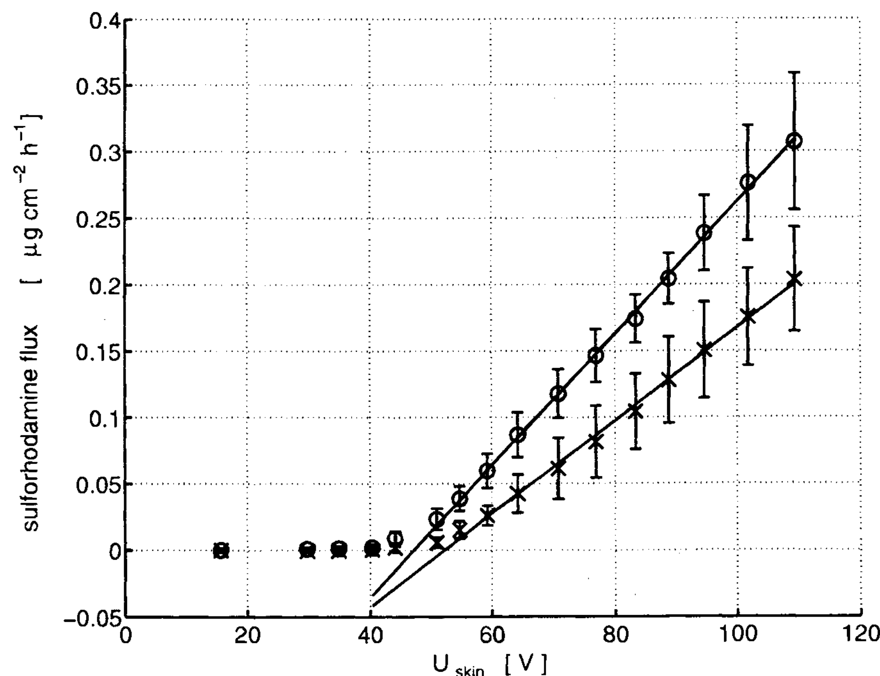

Fig. 3. Variation of the transdermal sulphorhodamine flux with the applied voltage in the presence (O) and absence (X) of ultrasound. Presented as means and S.D. of at least three repetitions.

Cavitation may play a two-fold role in enhancing the effect of electric field on transdermal transport. First, oscillations of cavitation bubbles induce partial structural disordering of the skin's lipid bilayers (4). Since the electrical resistance of the disordered bilayers is likely to be smaller than that of the normal lipid bilayers, the applied electric field may concentrate preferentially across the normal bilayers. This may decrease the threshold electroporating voltage for transdermal transport of calcein and sulphorhodamine. As mentioned in (see Figure 3), application of ultrasound reduces the threshold pulsing voltage from about 53 ± 3 V in the absence of ultrasound to about 46 ± 3 V in the presence of ultrasound (a reduction of about 12%). It is interesting that this number is comparable to an independent estimate of the fraction of SC bilayer disordered by ultrasound application (15%) (4).

The oscillations of cavitation bubbles may also induce convection across the skin. In order to assess the role of convection in the synergistic effect of ultrasound and electric field, we measured transdermal calcein and sulphorhodamine transport sequentially in the presence of electric field alone, ultrasound and electric field, ultrasound alone and in the absence of ultrasound and electric field. The results of these sequential procedures are shown in Figure 4. Results from a single experiment are shown to depict the shape of the curves clearly. Note the change in the transdermal flux at 1 and 1.5 hours when electric field and ultrasound is turned OFF respectively. If electrophoresis plays an important role in calcein and sulphorhodamine transport, the transdermal flux is likely to decrease rapidly after *electric fields* is turned OFF. On the other hand, if cavitation-induced convection plays an important role, transdermal flux would rapidly decrease after turning *ultrasound* OFF. Indeed, calcein flux decreases rapidly after turning electric field OFF (1 hour) and achieves a value comparable to the background flux (see Figure 4). When ultrasound is turned OFF at 1.5 hours, calcein flux further decreases by a small amount (compared to the reduction after turning electric field OFF at 1 hour) and thereafter it remains nearly at the background level. This suggests that calcein transport is mainly driven by electric forces. On the other hand, convection appears to play an important role in transdermal sulphorhodamine transport in the presence of ultrasound and electric field because the sulphorhodamine flux *did not decrease rapidly* after turning electric fields OFF, but decreased *instantaneously* after turning ultrasound OFF at 1.5 hours. Note that the total decrease in the transdermal sulphorhodamine flux after turning the electric field OFF (that is, between a period of 1 and 1.5 hours) is comparable to the instantaneous decrease in its value after turning ultrasound OFF at 1.5 hours. This suggests that both electric field and ultrasound-generated convection may play an important role in transdermal sulphorhodamine transport. This difference in the behavior of calcein and sulphorhodamine is conceivable because calcein possess a much larger charge (−4) compared to sulphorhodamine (−1). In this respect, it is important to note that the transdermal transport of calcein and sulphorhodamine in the presence of electric field alone also differs significantly. Calcein transport increases rapidly and achieves a steady state within 15 minutes. Sulphorhodamine flux, however, increases continuously with time over the experimental duration. This

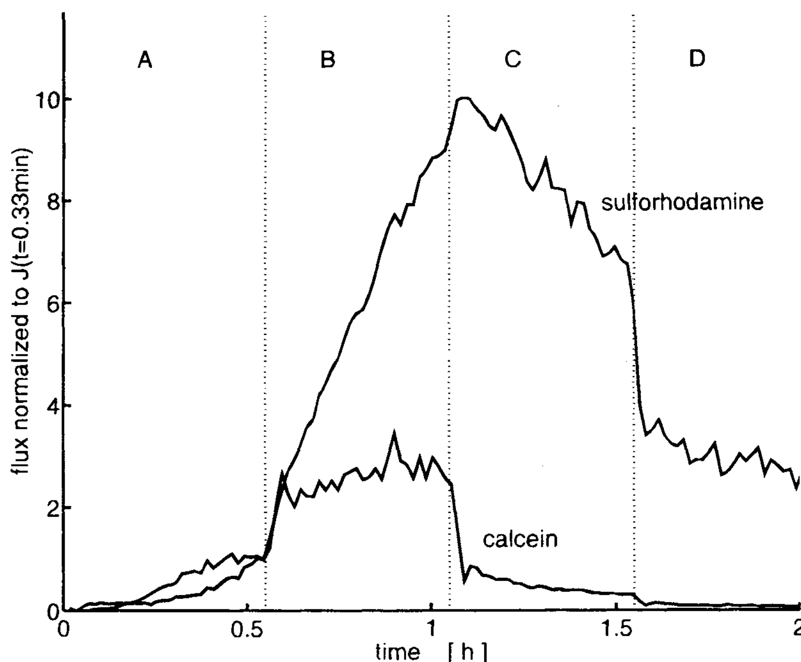

Fig. 4. Variation of the normalized transdermal calcein and sulphorhodamine flux under a variety of conditions: **A**- in the presence of electric field alone, **B**- in the presence of ultrasound and electric field, **C**- in the presence of ultrasound alone, **D**- in the absence of ultrasound and electric field. The transdermal calcein and sulphorhodamine fluxes have been normalized by the corresponding fluxes prior to application of ultrasound, that is, at the end of 0.5 hours. This was done to assist comparison of the relative changes in transdermal flux under different conditions.

difference in the behavior of calcein and sulphorhodamine flux may also be attributed to the lower charge on sulphorhodamine, as the transport during the electrical pulses is driven by electrophoresis.

ACKNOWLEDGMENTS

This work was supported by the National Institute of Health (NIH grant GM44884) (RL), United States-Israel Binational Science Foundation (BSF grant 9300244) (JK), Army Office Grant No. DAAL03-90-G0218, NIH Shannon Award (JW), Schering-Plough Foundation (SM), and Deutsche Forschunggemeinsschaft (UP).

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