Electronic Supplementary Material (ESI) for Soft Matter

Supporting Information

Experimental Section

Film preparation: Glass fibers (cross section: 100 µm, Hilgenberg, Germany) were first cleaned with 2% HellmanexII (Helma, Germany), then twice with 1 M HCl (Merck, Germany) and subsequently twice with millipore water. Cleaning steps were performed at 60 °C and under continuous stirring. For coating with polyelectrolyte multilayers, the glass fibers were alternately dipped into 0.5 mg ml^{-1} poly(L-lysine) (PLL, ~ 24 kDa, Sigma, Germany) and 0.5 mg ml^{-1} hyaluronic acid (HA, ~ 357 kDa, Lifecore Biomedical, USA) in 10 mM TRIS buffer (Sigma, Germany) with 15 mM NaCl (Sigma, Germany) (pH adjusted to 7.2) using a robot (DR 3, Riegler and Kirstein, Germany) for 10 min each. After each coating step, the fibers were rinsed thrice in TRIS buffer for 3 min. The dipping procedure was executed at 37 °C until 24 bilayers were reached.

Protein labelling: For determining their diffusion constants, the proteins papain (Sigma, Germany), lysozyme (Merck, Germany), alpha-lactalbumin (Sigma, Germany) and catalase (Sigma, Germany) were labelled with FITC (fluorescein isothiocyanate, Sigma, Germany) by following an established protocol.[34] Since this labelling requires another buffer, we subsequent dialysed the labelled proteins against TRIS buffer. The molar ratio of labelling was one fluorophore per five protein molecules. It was chosen this low to avoid effects on the of protein properties through the addition of the hydrophobic dye. Dextran (10 kDa and 500 kDa) and BSA were purchased in labelled form (all Sigma, Germany).

Determination of elastic modulus: Colloidal probe measurements were performed with an AFM (Nanowizard I, JPK Instruments, Germany) mounted on an optical microscope (IX51, Olympus, Japan) as described by Madaboosi *et al.*[18]

FRAP: After coating with PLL / HA, the fibers were incubated with the FITC-labelled molecules (papain, lysozyme, lactalbumin and catalase: 2 mg ml^{-1}, BSA: 0.5 mg ml^{-1}, FITC: 0.1 mg ml^{-1}) for 45 min at 22 °C under pH 7.2 and rinsed thrice with TRIS buffer. The last deposition step of film preparation was always performed with the polymer of opposite charge as that of the target molecules. *E. g.* for positively charged lysozyme, the last deposition step was negatively charged HA. For the FRAP experiments, a confocal laser scanning microscope (510 Meta, Zeiss, Germany) equipped with an argon laser and a 63 x / 1.4 oil immersion objective was employed. A 2 µm wide area extending perpendicular to the glass fiber was bleached with maximal laser power (Figure 1d). For image acquisition, the pinhole was set to 1 Airy unit (image slice of approximately 0.7 µm). The recovery of the FITC-labelled protein was detected every 2 s until the protein was homogenously distributed in the polyelectrolyte multilayer (at least for 60 s). Bleaching during scanning necessitated a time-dependent background correction. For that, the fluorescence intensity of FITC-labelled molecules was recorded in a control area using the same time interval as for FRAP but omitting the bleaching.

Determination of the diffusion time: To obtain the relative amount and position of the FITC-labelled molecules, a fluorescence intensity profile was measured perpendicular to the bleached rectangle in every recorded image (Image-Pro Plus, Media Cybernetics, USA). To reduce the noise, all intensity profiles were averaged along the PEM. The data was then background-corrected and normalized. Subsequently, the

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diffusion coefficient was calculated as described in the main text (Eqn. 1) using a purpose-made Mathematica 7.0 script (Wolfram Research, Champaign, IL).

Supporting Information Figure

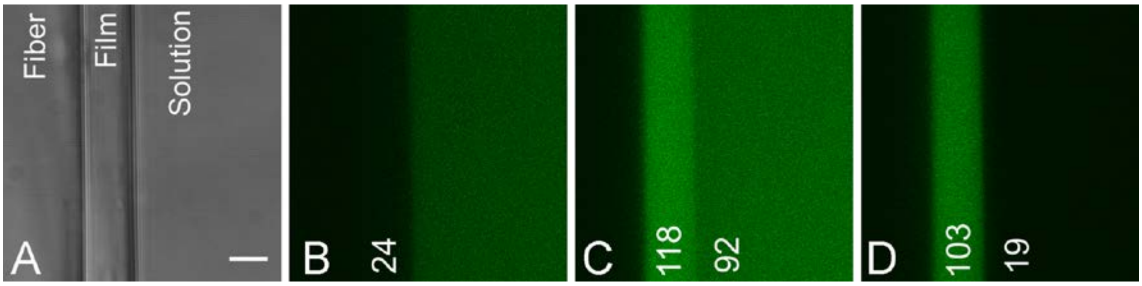

Fig. S1: A) Transmission image of a PEM-coated glass fiber in the buffer solution. B) Confocal cross section in the middle of the PEM fiber during incubation in fluorescently labelled lysozyme (green) after 0 min, C) after 45 min and D) after washing. The numbers give the fluorescence intensity in arbitrary units. After 45 min of incubation (C), the fluorescence intensity in the PEM even exceeds that in the solution. This accumulation indicates an immobilization of the protein. Washing with protein-free buffer (D) removes only approximately 12% of the protein from the PEM. Scale bar 10 µm.

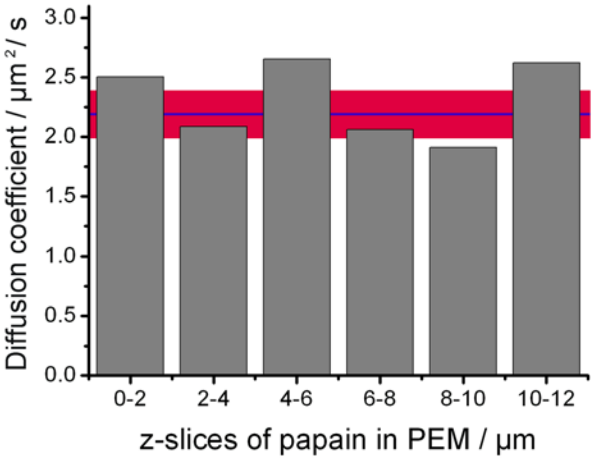

Fig. S2: Diffusion coefficient of papain in a polyelectrolyte multilayer (PEM). Averages of 2 µm sections along the film height (*z*, gray) in comparison with the total average over the whole film ((2.2 ± 0.2) µm^2 s^{-1}, blue).

Analytical solution of diffusion equation

In most reports on applications of the FRAP technique, only diffusion of the fluorescent species back into a spot bleached by using a laser beam of Gaussian intensity profile is considered. This, however, is inappropriate if the beam is scanned across a region for bleaching a larger area, *e. g.* by means of a confocal microscope. Such set-ups usually allow for scanning along three mutually perpendicular axes *x*, *y* and *z*, thus resulting in rectangular bleached regions. Since earlier publications implied that this problem can only be tackled numerically [Waharte 2010], we recapitulate the analytical solution for the 1d (line), 2d (square) and 3d (cube) case below. Analogous approaches for circular geometries (2d: circle, 3d: cylinder) described in polar coordinates, in contrast, lead to integrals of Bessel functions, for which we did not find analytical solutions. The calculus allows for imperfect bleaching, *i. e.* the concentration of fluorescent moieties is not zero but c_1 inside the bleached area immediately after bleaching (and c_2 outside of it). We do not consider diffusion during bleaching.

We aim to solve Fick's second law that describes the concentration c as a function of time t and spatial coordinate $\vec{x}$:

$$\frac{\partial c(\vec{x},t)}{\partial t} = a^2 \Delta c(\vec{x},t) \tag{1}$$

with the diffusion coefficient $D = a^2$. The *ansatz* is the same for all three cases (1d, 2d, 3d), but since the complexity increases, we start with 1d.

1d diffusion along a line

Then the initial value problem is defined by:

$$c(x,0) = \begin{cases} c_2 & \text{if} \quad x < x_1 \\ c_1 & \text{if} \quad x_1 \le x \le x_2 \\ c_2 & \text{if} \quad x_2 < x \end{cases} \tag{2}$$

We chose the coordinates so that w. l. o. g.

$$x_1 = -x_2 < 0 \tag{3}$$

We apply the 1d case of the Poisson integral for solving this initial value problem [Bronstein 1979]:

$$c(x,t)=\frac{1}{2a\sqrt{\pi}}\int_{-\infty}^{\infty}\frac{c(x',0)}{\sqrt{t}}\exp\left(-\frac{(x-x')^2}{4ta^2}\right)\mathrm{d}x' \tag{4}$$

Using Eqn. 3, this yields

$$\begin{aligned}c(x,t)=\frac{1}{2a\sqrt{\pi}}\Big[&\int_{-\infty}^{-x_2}\frac{c_2}{\sqrt{t}}\exp\left(-\frac{(x-x')^2}{4ta^2}\right)\mathrm{d}x'+\\&+\int_{-x_2}^{x_2}\frac{c_1}{\sqrt{t}}\exp\left(-\frac{(x-x')^2}{4ta^2}\right)\mathrm{d}x'+\\&+\int_{x_2}^{\infty}\frac{c_2}{\sqrt{t}}\exp\left(-\frac{(x-x')^2}{4ta^2}\right)\mathrm{d}x'\Big]\end{aligned} \tag{5}$$

Now we define a function A_0 as

$$A_0(x,t,x_0,c_1,c_2)=\int_{-\infty}^{-x_0}c_2\exp\left(-\frac{(x-x')^2}{4ta^2}\right)\mathrm{d}x'+\int_{-x_0}^{x_0}c_1\exp\left(-\frac{(x-x')^2}{4ta^2}\right)\mathrm{d}x'+\int_{x_0}^{\infty}c_2\exp\left(-\frac{(x-x')^2}{4ta^2}\right)\mathrm{d}x' \tag{6}$$

Then

$$c(x,t)=\frac{1}{2a\sqrt{\pi t}}A_0(x,t,x_2,c_1,c_2) \tag{7}$$

We introduce a substitution

$$\frac{x-x'}{2\sqrt{t}a}=y \quad\Rightarrow\quad \frac{\mathrm{d}y}{\mathrm{d}x'}=-\frac{1}{2\sqrt{t}a} \quad\Leftrightarrow\quad \mathrm{d}x'=-2\sqrt{t}a\,\mathrm{d}y \tag{8}$$

Hence, Eqn. 6 becomes

$$A_0(x,t,x_2,c_1,c_2)=-2\sqrt{t}a\left\{c_2\int_{\infty}^{\frac{x+x_2}{2\sqrt{t}a}}\mathrm{e}^{-y^2}\mathrm{d}y+c_1\int_{\frac{x+x_2}{2\sqrt{t}a}}^{\frac{x-x_2}{2\sqrt{t}a}}\mathrm{e}^{-y^2}\,\mathrm{d}y+c_2\int_{\frac{x-x_2}{2\sqrt{t}a}}^{-\infty}\mathrm{e}^{-y^2}\,\mathrm{d}y\right\} \tag{9}$$

Note that the limits had to be adjusted to the new variable of integration (y) using Eqn. 8. Now, we exchange the limits in first and second addend and use the substitution $y = -\tau$ in third addend:

$$A_0(x,t,x_2,c_1,c_2)=2\sqrt{t}a\left\{c_2\int_{\frac{x+x_2}{2\sqrt{t}a}}^{+\infty}\mathrm{e}^{-y^2}\mathrm{d}y+c_1\int_{\frac{x-x_2}{2\sqrt{t}a}}^{\frac{x+x_2}{2\sqrt{t}a}}\mathrm{e}^{-y^2}\,\mathrm{d}y+c_2\int_{-\frac{x-x_2}{2\sqrt{t}a}}^{\infty}\mathrm{e}^{-\tau^2}\,\mathrm{d}\tau\right\} \tag{10}$$

Next, we apply the complementary error function

$$\mathrm{erfc}(z)=1-\mathrm{erf}(z)=\frac{2}{\sqrt{\pi}}\int_z^{\infty}\mathrm{e}^{-\tau^2}\,\mathrm{d}\tau \tag{11}$$

and the generalized error function

$$\operatorname{erf}(a,b)=\operatorname{erf}(b)-\operatorname{erf}(a)=\frac{2}{\sqrt{\pi}}\int_a^b e^{-\tau^2}\,\mathrm{d}\tau \qquad (12)$$

to Eqn. 10, yielding

$$A_0(x,t,x_2,c_1,c_2)=\sqrt{\pi t}a\left\{c_2\operatorname{erfc}\left(\frac{x+x_2}{2\sqrt{ta}}\right)+c_1\operatorname{erf}\left(\frac{x-x_2}{2\sqrt{ta}},\frac{x+x_2}{2\sqrt{ta}}\right)+c_2\operatorname{erfc}\left(-\frac{x-x_2}{2\sqrt{ta}}\right)\right\} \qquad (13)$$

Using the properties of the complementary (Eqn. 11) and generalized (Eqn. 12) error function

$$A_0(x,t,x_2,c_1,c_2)=\sqrt{\pi t}a\left\{c_2\left[1-\operatorname{erf}\left(\frac{x+x_2}{2\sqrt{ta}}\right)\right]+c_1\left[\operatorname{erf}\left(\frac{x+x_2}{2\sqrt{ta}}\right)-\operatorname{erf}\left(\frac{x-x_2}{2\sqrt{ta}}\right)\right]+c_2\left[1-\operatorname{erf}\left(-\frac{x-x_2}{2\sqrt{ta}}\right)\right]\right\}$$
(14)

which after sorting reads:

$$A_0(x,t,x_2,c_1,c_2)=\sqrt{\pi t}a\left\{2c_2-c_2\left[\operatorname{erf}\left(\frac{x+x_2}{2\sqrt{ta}}\right)+\operatorname{erf}\left(-\frac{x-x_2}{2\sqrt{ta}}\right)\right]+c_1\left[\operatorname{erf}\left(\frac{x+x_2}{2\sqrt{ta}}\right)-\operatorname{erf}\left(\frac{x-x_2}{2\sqrt{ta}}\right)\right]\right\} \qquad (15)$$

Since the error function is odd,

$$A_0(x,t,x_2,c_1,c_2)=a\sqrt{\pi t}\left\{2c_2-c_2\left[\operatorname{erf}\left(\frac{x+x_2}{2\sqrt{ta}}\right)-\operatorname{erf}\left(\frac{x-x_2}{2\sqrt{ta}}\right)\right]+c_1\left[\operatorname{erf}\left(\frac{x+x_2}{2\sqrt{ta}}\right)-\operatorname{erf}\left(\frac{x-x_2}{2\sqrt{ta}}\right)\right]\right\} \qquad (16)$$

or, simplified,

$$A_0(x,t,x_2,c_1,c_2)=2a\sqrt{\pi t}\left\{c_2-\frac{c_2-c_1}{2}\left[\operatorname{erf}\left(\frac{x+x_2}{2\sqrt{ta}}\right)-\operatorname{erf}\left(\frac{x-x_2}{2\sqrt{ta}}\right)\right]\right\} \qquad (17)$$

Re-substitution into Eqn. 7 yields the 1d solution

$$c(x,t)=c_2-\frac{c_2-c_1}{2}\left[\operatorname{erf}\left(\frac{x+x_2}{2\sqrt{ta}}\right)-\operatorname{erf}\left(\frac{x-x_2}{2\sqrt{ta}}\right)\right] \qquad (18)$$

2 2d diffusion into a square

The square has an edge length of L. Then, the initial value problem is defined by:

$$c(x,y,0)=\begin{cases}c_2 & \text{if} \quad |x|>L/2 \;\vee\; |y|>L/2\\ c_1 & \text{if} \quad |x|\le L/2 \;\wedge\; |y|\le L/2\end{cases} \qquad (19)$$

We use the n-dimensional Poisson integral [Bronstein 1999]:

$$c(\vec{x},t)=\frac{1}{(2a\sqrt{\pi t})^n}\int c(\vec{x}',0)\exp\left(-\frac{(\vec{x}-\vec{x}')^2}{4ta^2}\right)\mathrm{d}V'$$
(20)

which, consequently, reads:

$$c(x,y,t)=\frac{1}{(2a\sqrt{\pi t})^2}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}c(x',y',0)\exp\left(-\frac{(x-x')^2+(y-y')^2}{4ta^2}\right)\mathrm{d}y'\mathrm{d}x' \tag{21}$$

$$c(x,y,t)=\frac{1}{(2a\sqrt{\pi t})^2}\int_{-\infty}^{\infty}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\int_{-\infty}^{\infty}c(x',y',0)\,\mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\mathrm{d}y'\mathrm{d}x' \tag{22}$$

Let us define A_1 as

$$A_1(y,t,x')=\int_{-\infty}^{\infty}c(x',y',0)\,\mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\mathrm{d}y' \tag{23}$$

Now two cases have to be distinguished:

1. If $|x'|\leq L/2$ then

$$A_1(y,t,x')=\int_{-\infty}^{-L/2}c_2\,\mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\mathrm{d}y'+\int_{-L/2}^{L/2}c_1\,\mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\mathrm{d}y'+\int_{L/2}^{\infty}c_2\,\mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\mathrm{d}y' \tag{24}$$

which, using Eqn. 6, is

$$A_1(y,t,x')=A_0(y,t,L/2,c_1,c_2) \tag{25}$$

2. If $|x'| > L/2$, then

$$A_1(y,t,x')=\int_{-\infty}^{\infty}c_2\,\mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\mathrm{d}y' \tag{26}$$

With the substitution

$$\frac{y-y'}{2\sqrt{t}a}=\tau \quad\Rightarrow\quad \frac{\mathrm{d}\tau}{\mathrm{d}y'}=-\frac{1}{2\sqrt{t}a} \quad\Leftrightarrow\quad \mathrm{d}y'=-2\sqrt{t}a\,\mathrm{d}\tau \tag{27}$$

and using Eqn. 11,

$$\begin{aligned}A_1(y,t,x')&=\int_{\infty}^{-\infty}c_2\exp(-\tau^2)(-2\sqrt{t}a)\mathrm{d}\tau\\&=c_2a\sqrt{t}\int_{-\infty}^{\infty}\exp(-\tau^2)\mathrm{d}\tau\\&=c_2a\sqrt{\pi t}\,\mathrm{erfc}(-\infty)\\&=c_2a\sqrt{\pi t}[1-\mathrm{erf}(-\infty)]\end{aligned} \tag{28}$$

Since

$$\lim_{x\to\infty}\mathrm{erf}(x)=1 \tag{29}$$

$$A_1(y,t,x')=2c_2a\sqrt{\pi t} \tag{30}$$

For the Poisson integral (Eqn. 22) follows

$$c(x,y,t)=\frac{1}{(2a\sqrt{\pi t})^2}\int_{-\infty}^{\infty}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\,A_1(y,t,x')\,\mathrm{d}x' \tag{31}$$

$$\begin{aligned}c(x,y,z,t)&=\frac{1}{(2a\sqrt{\pi t})^2}\left\{\int_{-\infty}^{-L/2}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\,2c_2a\sqrt{\pi t}\,\mathrm{d}x'+\int_{-L/2}^{L/2}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\,A_0(y,t,L/2,c_1,c_2)\,\mathrm{d}x'+\int_{L/2}^{\infty}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\,2c_2a\sqrt{\pi t}\,\mathrm{d}x'\right\}\\&=\frac{1}{(2a\sqrt{\pi t})^2}\left\{2c_2a\sqrt{\pi t}\int_{-\infty}^{-L/2}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\,\mathrm{d}x'+A_0(y,t,L/2,c_1,c_2)\int_{-L/2}^{L/2}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\,\mathrm{d}x'+2c_2a\sqrt{\pi t}\int_{L/2}^{\infty}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\,\mathrm{d}x'\right\}\end{aligned} \tag{32}$$

Using again the definition of A_0 (Eqn. 6) results in

$$c(x,y,z,t)=\frac{1}{(2a\sqrt{\pi t})^2}A_0(x,t,L/2,A_0(y,t,L/2,c_1,c_2),2c_2a\sqrt{\pi t}) \tag{33}$$

Successively resubstituting A_0 using Eqn. 17 and

$$\Delta\mathrm{erf}(x)=\mathrm{erf}\,\frac{x+L/2}{2\sqrt{ta}}-\mathrm{erf}\,\frac{x-L/2}{2\sqrt{ta}} \tag{34}$$

gives

$$\begin{aligned}c(x,y,z,t)&=\frac{1}{(2a\sqrt{\pi t})^2}2a\sqrt{\pi t}\left\{2c_2a\sqrt{\pi t}-\frac{2c_2a\sqrt{\pi t}-A_0(y,t,L/2,c_1,c_2)}{2}\Delta\mathrm{erf}(x)\right\}\\&=c_2-\frac{c_2-\frac{A_0(y,t,L/2,c_1,c_2)}{2a\sqrt{\pi t}}}{2}\Delta\mathrm{erf}(x)\\&=c_2-\frac{c_2-\frac{2a\sqrt{\pi t}\left\{c_2-\frac{c_2-c_1}{2}\Delta\mathrm{erf}(y)\right\}}{2a\sqrt{\pi t}}}{2}\Delta\mathrm{erf}(x)\\&=c_2-\frac{c_2-c_2+\frac{c_2-c_1}{2}\Delta\mathrm{erf}(y)}{2}\Delta\mathrm{erf}(x)\\&=c_2-\frac{c_2-c_1}{4}\Delta\mathrm{erf}(y)\,\Delta\mathrm{erf}(x)\end{aligned} \tag{35}$$

and finally the 2d solution

$$c(x,y,t)=c_2-(c_2-c_1)\frac{\left[\mathrm{erf}\left(\frac{y+L/2}{2\sqrt{ta}}\right)-\mathrm{erf}\left(\frac{y-L/2}{2\sqrt{ta}}\right)\right]\left[\mathrm{erf}\left(\frac{x+L/2}{2\sqrt{ta}}\right)-\mathrm{erf}\left(\frac{x-L/2}{2\sqrt{ta}}\right)\right]}{4} \tag{36}$$

3d diffusion into a cube

The initial value problem is defined by:

$$c(x,y,z,0)=\begin{cases} c_2 & \text{if} \quad |x|>L/2 \quad \vee \quad |y|>L/2 \quad \vee \quad |z|>L/2 \\ c_1 & \text{if} \quad |x|\le L/2 \quad \wedge \quad |y|\le L/2 \quad \wedge \quad |z|\le L/2 \end{cases} \tag{37}$$

The Poisson integral (Eqn. 20) then becomes:

$$c(x,y,z,t)=\frac{1}{(2a\sqrt{\pi t})^3}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} c(x',y',z',0)\exp\left(-\frac{(x-x')^2+(y-y')^2+(z-z')^2}{4ta^2}\right)\mathrm{d}z'\,\mathrm{d}y'\,\mathrm{d}x' \tag{38}$$

$$c(x,y,z,t)=\frac{1}{(2a\sqrt{\pi t})^3}\int_{-\infty}^{\infty} \mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\int_{-\infty}^{\infty} \mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\int_{-\infty}^{\infty} c(x',y',z',0)\,\mathrm{e}^{-\frac{(z-z')^2}{4ta^2}}\,\mathrm{d}z'\,\mathrm{d}y'\,\mathrm{d}x' \tag{39}$$

We define A_1 as

$$A_1(z,t,x',y')=\int_{-\infty}^{\infty} c(x',y',z',0)\,\mathrm{e}^{-\frac{(z-z')^2}{4ta^2}}\,\mathrm{d}z' \tag{40}$$

Now two cases have to be distinguished:

1. If $|x'|\le L/2 \wedge |y'|\le L/2$, then

$$A_1(z,t,x',y')=\int_{-\infty}^{-L/2} c_2\,\mathrm{e}^{-\frac{(z-z')^2}{4ta^2}}\,\mathrm{d}z'+\int_{-L/2}^{L/2} c_1\,\mathrm{e}^{-\frac{(z-z')^2}{4ta^2}}\,\mathrm{d}z'+\int_{L/2}^{\infty} c_2\,\mathrm{e}^{-\frac{(z-z')^2}{4ta^2}}\,\mathrm{d}z' \tag{41}$$

or, using Eqn. 6,

$$A_1(z,t,x',y')=A_0(z,t,L/2,c_1,c_2) \tag{42}$$

2. If $|x'|>L/2 \vee |y'|>L/2$, then

$$A_1(z,t,x',y')=\int_{-\infty}^{\infty} c_2\,\mathrm{e}^{-\frac{(z-z')^2}{4ta^2}}\,\mathrm{d}z' \tag{43}$$

With the substitution

$$\frac{z-z'}{2\sqrt{t}a}=\tau \quad \Rightarrow \quad \frac{\mathrm{d}\tau}{\mathrm{d}z'}=-\frac{1}{2\sqrt{t}a} \quad \Leftrightarrow \quad \mathrm{d}z'=-2\sqrt{t}a\,\mathrm{d}\tau \tag{44}$$

$$A_1(z,t,x',y')=\int_{\infty}^{-\infty} c_2\exp(-\tau^2)(-2\sqrt{t}a)\,\mathrm{d}\tau$$
$$=c_2 a 2\sqrt{t}\int_{-\infty}^{\infty}\exp(-\tau^2)\,\mathrm{d}\tau$$

(45)

Using Eqn. 11

$$A_1(z,t,x',y') = c_2 a\sqrt{\pi t}\,\mathrm{erfc}(-\infty) \\ = c_2 a\sqrt{\pi t}\,[1-\mathrm{erf}(-\infty)] \quad (46)$$

and Eqn. 29

$$A_1(z,t,x',y') = 2c_2 a\sqrt{\pi t} \quad (47)$$

For the Poisson integral (Eqn. 39) follows

$$c(x,y,z,t) = \frac{1}{(2a\sqrt{\pi t})^3}\int_{-\infty}^{\infty} \mathrm{e}^{-\frac{(x-x')^2}{4ta^2}} \int_{-\infty}^{\infty} \mathrm{e}^{-\frac{(y-y')^2}{4ta^2}} A_1(z,t,x',y')\,\mathrm{d}y'\,\mathrm{d}x' \quad (48)$$

Let us define:

$$A_2(y,z,t,x') = \int_{-\infty}^{\infty} \mathrm{e}^{-\frac{(y-y')^2}{4ta^2}} A_1(z,t,x',y')\,\mathrm{d}y' \quad (49)$$

Again two cases have to be distinguished:

1. If $|x'| \le L/2$, then with Eqn. 42 and 47

$$A_2(y,z,t,x') = \int_{-\infty}^{-L/2} 2c_2 a\sqrt{\pi t}\,\mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\,\mathrm{d}y' + \int_{-L/2}^{L/2} \mathrm{e}^{-\frac{(y-y')^2}{4ta^2}} A_0(z,t,L/2,c_1,c_2)\,\mathrm{d}y' + \int_{L/2}^{\infty} 2c_2 a\sqrt{\pi t}\,\mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\,\mathrm{d}y' \quad (50)$$

$$A_2(y,z,t,x') = 2c_2 a\sqrt{\pi t}\int_{-\infty}^{-L/2} \mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\,\mathrm{d}y' + A_0(z,t,L/2,c_1,c_2)\int_{-L/2}^{L/2} \mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\,\mathrm{d}y' + 2c_2 a\sqrt{\pi t}\int_{L/2}^{\infty} \mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\,\mathrm{d}y' \quad (51)$$

With the definition of A_0 (Eqn. 6) follows

$$A_2(y,z,t,x') = A_0(y,t,L/2,A_0(z,t,L/2,c_1,c_2),2c_2 a\sqrt{\pi t}) \quad (52)$$

2. If $|x'| > L/2$, then with Eqn. 47

$$A_2(y,z,t,x') = \int_{-\infty}^{\infty} 2c_2 a\sqrt{\pi t}\,\mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\,\mathrm{d}y' = 2c_2 a\sqrt{\pi t}\int_{-\infty}^{\infty} \mathrm{e}^{-\frac{(y-y')^2}{4ta^2}}\,\mathrm{d}y' \quad (53)$$

with the substitution Eqn. 27

$$A_2(y,z,t,x') = 2c_2 a\sqrt{\pi t}\int_{\infty}^{-\infty} \mathrm{e}^{-\tau^2}\left(-2\sqrt{t}a\right)\mathrm{d}\tau \\ = 2c_2 ta^2 2\sqrt{\pi}\int_{-\infty}^{\infty} \mathrm{e}^{-\tau^2}\,\mathrm{d}\tau \quad (54)$$

and using Eqn. 11

$$A_2(y,z,t,x') = 2c_2 ta^2\pi\,\mathrm{erfc}(-\infty) \\ = 2c_2 ta^2\pi[1-\mathrm{erf}(-\infty)] \quad (55)$$

and Eqn. 29

$$A_2\left(y,z,t,x'\right)=4c_2ta^2\pi \tag{56}$$

Inserting the results for A_2 into Eqn. 48 gives

$$c\left(x,y,z,t\right)=\frac{1}{(2a\sqrt{\pi t})^3}\int_{-\infty}^{\infty}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}A_2(y,z,t,x')\,\mathrm{d}x' \tag{57}$$

$$\begin{aligned}c\left(x,y,z,t\right)=\frac{1}{(2a\sqrt{\pi t})^3}\Bigg[&\int_{-\infty}^{-L/2}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}4c_2ta^2\pi\,\mathrm{d}x'+\\&+\int_{-L/2}^{L/2}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}A_0(y,t,L/2,A_0(z,t,L/2,c_1,c_2),2c_2a\sqrt{\pi t})\,\mathrm{d}x'+\\&+\int_{L/2}^{\infty}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}4c_2ta^2\pi\,\mathrm{d}x'\Bigg]\\=\frac{1}{(2a\sqrt{\pi t})^3}\Bigg[&4c_2ta^2\pi\int_{-\infty}^{-L/2}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\,\mathrm{d}x'+\\&+A_0(y,t,L/2,A_0(z,t,L/2,c_1,c_2),2c_2a\sqrt{\pi t})\int_{-L/2}^{L/2}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\,dx'+\\&+4c_2ta^2\pi\int_{L/2}^{\infty}\mathrm{e}^{-\frac{(x-x')^2}{4ta^2}}\,\mathrm{d}x'\Bigg]\end{aligned}$$

(58)

Using again the definition of A_0 (Eqn. 6) results in

$$c\left(x,y,z,t\right)=\frac{1}{(2a\sqrt{\pi t})^3}A_0(x,t,L/2,A_0(y,t,L/2,A_0(z,t,L/2,c_1,c_2),2c_2a\sqrt{\pi t}),4c_2ta^2\pi) \tag{59}$$

Successively re-substituting A_0 using Eqn. 17 and Eqn. 34 gives

$$
\begin{aligned}
c(x,y,z,t) &= \frac{1}{(2a\sqrt{\pi t})^3} 2a\sqrt{\pi t}\left\{4c_2 ta^2\pi - \frac{4c_2 ta^2\pi - A_0(y,t,\tfrac{L}{2},A_0(z,t,\tfrac{L}{2},c_1,c_2),2c_2 a\sqrt{\pi t})}{2}\Delta\mathrm{erf}(x)\right\} \\
&= c_2 - \frac{c_2 - \dfrac{A_0(y,t,\tfrac{L}{2},A_0(z,t,\tfrac{L}{2},c_1,c_2),2c_2 a\sqrt{\pi t})}{4ta^2\pi}}{2}\Delta\mathrm{erf}(x) \\
&= c_2 - \frac{c_2 - \dfrac{2a\sqrt{\pi t}\left\{2c_2 a\sqrt{\pi t} - \dfrac{2c_2 a\sqrt{\pi t} - A_0(z,t,\tfrac{L}{2},c_1,c_2)}{2}\Delta\mathrm{erf}(y)\right\}}{4ta^2\pi}}{2}\Delta\mathrm{erf}(x) \\
&= c_2 - \frac{c_2 - c_2 + \dfrac{c_2 - \dfrac{A_0(z,t,\tfrac{L}{2},c_1,c_2)}{2a\sqrt{\pi t}}2}{2}\Delta\mathrm{erf}(y)}{2}\Delta\mathrm{erf}(x) \\
&= c_2 - \frac{c_2 - \dfrac{A_0(z,t,\tfrac{L}{2},c_1,c_2)}{2a\sqrt{\pi t}}}{4}\Delta\mathrm{erf}(y)\,\Delta\mathrm{erf}(x) \\
&= c_2 - \frac{c_2 - \dfrac{2a\sqrt{\pi t}\left\{c_2 - \dfrac{c_2 - c_1}{2}\Delta\mathrm{erf}(z)\right\}}{2a\sqrt{\pi t}}}{4}\Delta\mathrm{erf}(y)\,\Delta\mathrm{erf}(x) \\
&= c_2 - \frac{c_2 - c_2 + \dfrac{c_2 - c_1}{2}\Delta\mathrm{erf}(z)}{4}\Delta\mathrm{erf}(y)\,\Delta\mathrm{erf}(x) \\
&= c_2 - \frac{c_2 - c_1}{8}\Delta\mathrm{erf}(z)\,\Delta\mathrm{erf}(y)\,\Delta\mathrm{erf}(x)
\end{aligned}
\tag{60}
$$

And after re-substituting according to Eqn. 34

$$
c(x,y,z,t) = c_2 - (c_2 - c_1)\frac{\left[\mathrm{erf}\left(\frac{z+\frac{L}{2}}{2\sqrt{ta}}\right) - \mathrm{erf}\left(\frac{z-\frac{L}{2}}{2\sqrt{ta}}\right)\right]\left[\mathrm{erf}\left(\frac{y+\frac{L}{2}}{2\sqrt{ta}}\right) - \mathrm{erf}\left(\frac{y-\frac{L}{2}}{2\sqrt{ta}}\right)\right]\left[\mathrm{erf}\left(\frac{x+\frac{L}{2}}{2\sqrt{ta}}\right) - \mathrm{erf}\left(\frac{x-\frac{L}{2}}{2\sqrt{ta}}\right)\right]}{8}
\tag{61}
$$

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