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# Generating isomorphous heavy-atom derivatives by a quick-soak method. Part I: test cases

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Screening for heavy-atom derivatives remains a time-consuming and cumbersome process that often results in non-isomorphous derivatives whose phases cannot be combined. Using lysozyme and Fc$\gamma$RIII receptor crystals as test cases, an improved soaking method for the generation of conventional heavy-atom derivatives has been developed. The method is based on soaking crystals in heavy-atom compounds for a very brief time at near-saturation concentrations. Compared with the current heavy-atom soaking method, which often takes days to achieve a derivatization, the quick-soak method completes a derivatization within 10 min to 2 h. The bound heavy-atom sites display higher peak heights from quick soaks than from overnight soaks in all cases tested. The quick-soak derivatives also preserved native-like diffraction resolution and data quality that was better than the prolonged-soak derivatives. Furthermore, derivatives generated by brief soaks are more isomorphous to the native than those generated by overnight soaks. Short soaks not only increase the likelihood of success in heavy-atom screening by reducing the pitfalls associated with prolonged soaks, such as lack of isomorphism and overall lattice disorder, but also have the potential to transform a time-consuming derivative screening into an 'on-the-fly' real-time derivatization process.

## 1. Introduction

Heavy-atom phasing remains the method of choice in solving new protein crystal structures. Prior to the use of synchrotron radiation, phases were calculated primarily using the multiple isomorphous replacement (MIR) method, which requires a lengthy screening process to identify suitable heavy-atom derivatives. With the application of tunable-wavelength synchrotron radiation, the multiwavelength anomalous dispersion (MAD) method quickly became the preferred phasing method owing to the fact that MAD phases are not subject to errors introduced by non-isomorphism. The introduction of selenomethionine (SeMet) MAD phasing eliminated the need to screen for heavy-atom derivatives and greatly facilitated X-ray phase determinations for many structures (Hendrickson, 1991). The SeMet MAD method, however, also has its shortcomings. For example, the method is mostly limited to recombinant bacterial expression systems, as no suitable method is available to express SeMet proteins in mammalian expression systems. It also becomes technically difficult to apply SeMet phasing to very large protein structures owing to the relatively small anomalous signal of Se compared with those of conventional heavy metals. Recently, a halide-based soaking method has been successfully applied to phase a number of small and well diffracting protein

**Table 1**
Data collection and phasing statistics of lysozyme derivatives.

Values in parentheses are for the highest resolution shell.

| | Native | $KAuCl_4$ | | | $K_2PtCl_6$ | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Data collection | | | | | | | | | |
| HA conc.† (m*M*) | | 10 | 10 | 1 | 10 | 12.3 | 1 | 1 | 10 |
| Soak time | | 10 min | 24 h | 48 h | 10 min | 10 min | 10 min | 22 h | 48 h |
| Unit-cell parameters (Å) | | | | | | | | | |
| *a*, *b* | 78.6 | 78.8 | 79.2 | 79.0 | 78.9 | 78.9 | 78.3 | 78.6 | 78.2 |
| *c* | 36.9 | 36.9 | 35.0 | 36.8 | 36.9 | 36.9 | 36.8 | 36.9 | 36.8 |
| Resolution (Å) | 1.75 | 1.78 | 3.24 | 1.78 | 1.78 | 1.78 | 1.78 | 1.78 | 1.92 |
| Completeness (%) | 97.1 | 93.6 | 92.9 | 89.3 | 99.3 | 95.8 | 97.1 | 89.7 | 98.5 |
| $R_{sym}$ | 0.056 (0.22) | 0.051 (0.11) | 0.138 (0.77) | 0.092 (0.58) | 0.044 (0.073) | 0.057 (0.074) | 0.058 (0.19) | 0.057 (0.22) | 0.074 (0.60) |
| $I/\sigma$ | 55.4 (15.3) | 46.5 (23.2) | 22.7 (3.1) | 28.2 (2.9) | 47.6 (33.0) | 53.1 (36.9) | 53.0 (17.1) | 55.8 (15.0) | 35.9 (4.2) |
| $R_{iso}$ | | 0.201 | 0.462 | 0.349 | 0.176 | 0.208 | 0.111 | 0.087 | 0.213 |
| Heavy-atom peak height‡ | | | | | | | | | |
| Site 1 ($\sigma$) | | 21.6 | <4.0 | 15.7 | 19.3 | 18.2 | <5.0 | 15.0 | 6.2 |
| Site 2 ($\sigma$) | | 12.8 | <4.0 | 9.3 | 16.3 | 16.5 | <5.0 | 10.5 | 11.0 |
| Site 3 ($\sigma$) | | 9.7 | <4.0 | 5.4 | | | | | |

† Heavy-atom soaking concentration. ‡ For the $KAuCl_4$ derivative, the coordinates for the three bound Au sites are (−11.36, 11.72, 19.21), (−8.49, 10.2, 14.25) and (3.30, 7.94, 9.84) Å, for sites 1, 2 and 3, respectively. Site 1 and 2 are bound to His15 and Arg14, respectively. The heavy-atom sites are shown as peak heights in standard deviation from the difference Fourier ($F_{PH} - F_P$) map. For the $K_2PtCl_6$ derivative, site 1 is bound to Arg14 with coordinates of (−10.96, 10.96, 9.23) and site 2 is close to Gln41 and Asn65 with coordinates of (6.14, 3.86, 29.99).

structures (Dauter *et al.*, 2000). In contrast to all other methods, the halide-soaking method is a true real-time derivatization method. This is particularly advantageous in the era of structural genomics. However, MIR remains the only viable phasing method for many large proteins and less well diffracting crystals. Unfortunately, MIR phasing is often exacerbated by the lack of isomorphism and by the difficulty in finding diffracting derivatives. To circumvent these potential pitfalls associated with MIR phasing, we now introduce a quick-soaking method for conventional heavy-atom derivative screening and phasing. Using hen egg-white lysozyme and Fc receptor crystals as test cases, we show that quick soaks generated better derivatives than overnight soaks as judged by the diffraction resolution, heavy-atom phasing occupancies, lack of isomorphism and other phasing statistics.

## 2. Materials and methods

### 2.1. Crystallization and data collection

Hen egg-white lysozyme (Sigma) at a concentration of 10 mg ml$^{-1}$ was used to grow the tetragonal crystal form by the hanging-drop method in 7% NaCl, 0.20 *M* sodium acetate buffer pH 4.7 (Alderton *et al.*, 1945). Fc$\gamma$RIII crystals were grown from 10% PEG 8000, 50 m*M* Na HEPES pH 7.0 and 7 mg ml$^{-1}$ protein concentration (Zhang *et al.*, 2000). Heavy-atom solutions were prepared in hanging-drop reservoir solutions and the soaks were carried out for specified time durations and terminated by flash-freezing under a liquid-nitrogen stream. The cryoprotectant solutions consisted of crystallization solutions with 25% glycerol. Native and derivative X-ray diffraction data sets were collected using an R-AXIS IV++ detector and processed using *HKL*2000 (Otwinowski & Minor, 1997). Heavy-atom binding sites were located by difference Fourier synthesis with ($F_{PH} - F_P$) as the amplitudes and phases calculated from the structural coordinates using *CNS* (Brünger *et al.*, 1998). Heavy-atom derivative phasing and statistics were calculated using *MLPHARE* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

### 2.2. Mass-spectrometry measurement of heavy-atom reaction kinetics

The reaction kinetics for heavy-atom derivatization of Fc$\gamma$RIII were monitored by mass-spectrometry measurements. The reactions were initiated by mixing 10 µl of 5 mg ml$^{-1}$ Fc$\gamma$RIII with 0.1% saturated $HgCl_2$ in 50 m*M* HEPES at pH 7.0. The conditions for the solution reactions resembled those used for heavy-atom soaking. To determine the reaction kinetics, aliquots of the reaction mixture were sampled and quenched by adding 50 m*M* L-histidine to the aliquots at specific time points. The samples were analyzed by electrospray ionization mass spectrometry as described previously (Sun & Hammer, 2000).

## 3. Results and discussion

### 3.1. Test case 1: derivatives of lysozyme crystals

Two previously known derivatives of lysozyme, $KAuCl_4$ and $K_2PtCl_6$, were chosen to test the length of soaking and heavy-metal concentrations. Both derivatives were originally obtained by soaking lysozyme crystals in $KAuCl_4$ and $K_2PtCl_6$ for 7–14 d (Blake, 1968). For brief derivatization, the crystals were soaked in a 10 m*M* concentration of heavy-atom solutions for 10 min, designated hereafter as a (10 m*M*, 10 min) soak. As a comparison, data were also collected from conventional 1–2 d soaked crystals.

The data for $KAuCl_4$ derivatives were initially collected under two different soaking conditions, (10 m*M*, 10 min) and (10 m*M*, 24 h). Since the 24 h soaking in 10 m*M* $KAuCl_4$ resulted in a marked reduction in the diffraction resolution, a

third data set was collected from a crystal soaked in 1 m*M* $KAuCl_4$ for 48 h (Table 1). The crystals subjected to the 10 min soak produced diffraction data similar in quality to native data as judged by diffraction resolution, $R_{sym}$ and $I/\sigma$ for the outermost resolution shell of reflections. In contrast, both the 24 and 48 h soaked crystals diffracted more weakly compared with the 10 min soaked crystal. When the derivative data were scaled against the native data, the isomorphous *R* factor ($R_{iso}$) from the 10 min, 24 and 48 h soaked crystals were 0.20, 0.46 and 0.34, respectively. The binding of $AuCl_4^-$ in the quick-soak derivative is evident in the difference Fourier ($F_{PH} - F_P$) map phased with the lysozyme coordinates (PDB entry 193l) (Fig. 1*a*). Interestingly, the intensity of gold binding, as indicated by the peak heights in the difference Fourier map, is inversely correlated to the $R_{iso}$ among the three derivatizations, with the quick soak resulting in the highest heavy-atom peak height (Table 1; Figs. 1*a*–1*c*). Similarly, the difference Patterson map from the 10 min soak also displays highest Patterson peaks among the three derivatives (Fig. 2). While lengthy derivatization reactions ought to result in more heavy-metal attachment, the observed inverse correlation between the soaking time and Au peak heights indicates an increase in the non-isomorphism accompanied with the longer soaks. Alternatively, non-specific heavy-atom binding present in the longer soaks could cause higher noise levels in their difference Fourier and Patterson maps, hence reducing the Au peak heights.

Five different $K_2PtCl_6$ soaking data sets were collected using similar size lysozyme crystals under the respective soaking conditions of (1 m*M*, 10 min), (1 m*M*, 22 h), (10 m*M*, 10 min), (10 m*M*, 48 h) and (12.3 m*M*, 10 min) (Table 1). $K_2PtCl_6$ saturates at a concentration of 12.3 m*M* in the current lysozyme crystallization solution. Similar to the $KAuCl_4$ derivative, the 10 min $K_2PtCl_6$ soak resulted in no deterioration in diffraction compared with the native data, whereas the 22 and 48 h soaks resulted in weaker diffraction as judged by

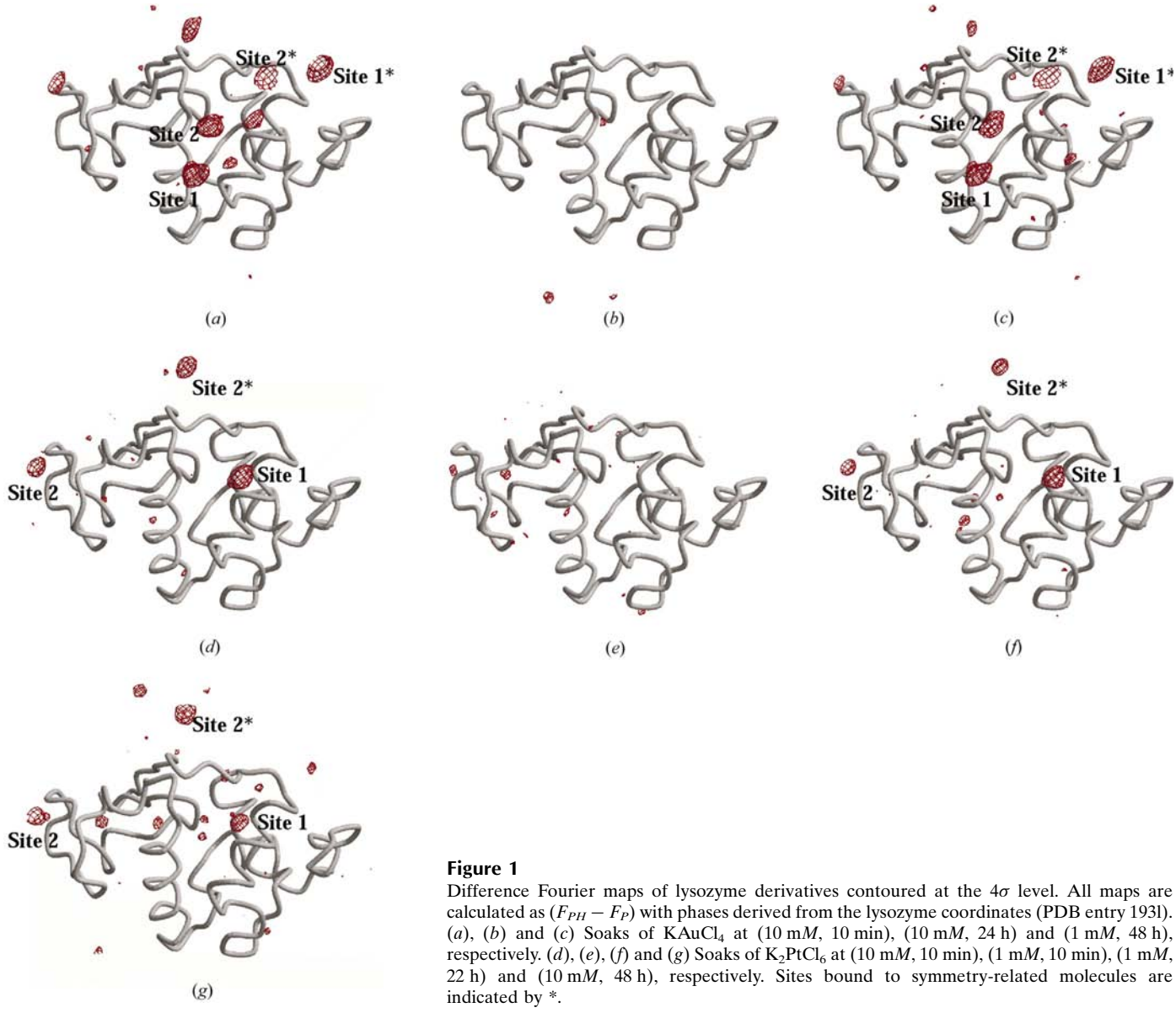


**Figure 1**
Difference Fourier maps of lysozyme derivatives contoured at the 4$\sigma$ level. All maps are calculated as ($F_{PH} - F_P$) with phases derived from the lysozyme coordinates (PDB entry 193l). (*a*), (*b*) and (*c*) Soaks of $KAuCl_4$ at (10 m*M*, 10 min), (10 m*M*, 24 h) and (1 m*M*, 48 h), respectively. (*d*), (*e*), (*f*) and (*g*) Soaks of $K_2PtCl_6$ at (10 m*M*, 10 min), (1 m*M*, 10 min), (1 m*M*, 22 h) and (10 m*M*, 48 h), respectively. Sites bound to symmetry-related molecules are indicated by *.

**Table 2**
Data collection and phasing statistics of Fc$\gamma$RIII derivative.

| | Native | TMLA | | | $HgCl_2$ | | |
|---|---|---|---|---|---|---|---|
| Data collection | | | | | | | |
| HA conc. (m*M*) | | 5 | 10 | 10 | Saturated | Saturated | Saturated |
| Soak time | | 10 min | 10 min | 24 h | 10 min | 2 h | >4 h |
| Unit-cell parameters (Å) | | | | | | | |
| *a* | 67.4 | 67.4 | 67.4 | 67.5 | 67.4 | 67.7 | |
| *b* | 85.9 | 85.6 | 86.0 | 85.6 | 85.6 | 86.5 | |
| *c* | 36.3 | 36.3 | 36.4 | 36.2 | 36.2 | 36.0 | |
| Resolution (Å) | 1.85 | 1.8 | 1.9 | 2.3 | 2.0 | 2.4 | ~8 |
| Completeness (%) | 99.9 | 96.7 | 90.8 | 98.0 | 97.1 | 97.9 | |
| $R_{sym}$ | 0.078 (0.44) | 0.086 (0.46) | 0.086 (0.41) | 0.11 (0.54) | 0.061 (0.35) | 0.069 (0.38) | |
| $I/\sigma$ | 23.5 (3.9) | 22.0 (3.0) | 15.2 (2.7) | 17.5 (3.6) | 30.0 (4.4) | 27.9 (4.2) | |
| $R_{iso}$ | | 0.093 | 0.09 | 0.168 | 0.119 | 0.273 | |
| Heavy-atom peak height† | | | | | | | |
| Site 1 ($\sigma$) | | 6.7 | 17.8 | <5.0 | 7.6 | 24.4 | |
| Site 2 ($\sigma$) | | 6.0 | 12.8 | <5.0 | 5.2 | 16.4 | |

† All heavy-atom sites are shown as peak heights in standard deviations from a difference Fourier ($F_{PH} - F_P$) map. The coordinates for site 1 and 2 of the TMLA-derivatized Fc$\gamma$RIII are (111.99, 12.54, 13.41) and (88.49, 21.42, 23.78), respectively. The coordinates for site 1 and 2 of the $HgCl_2$ derivatives are (80.27, 1.80, 27.71) and (104.52, 7.78, 29.52), respectively.

$R_{sym}$ and $I/\sigma$ for the outer most resolution shell. The (10 m*M*, 10 min) $K_2PtCl_6$ soak produced the highest platinum binding peaks in the difference Fourier maps (Table 1, Figs. 1*d*–1*g*). When the data from the three 10 min soaks with 1, 10 and 12.3 m*M* $K_2PtCl_6$, respectively, were compared, the results showed a significantly weaker binding of Pt in the 1 m*M* soak compared with the 10 and 12.3 m*M* soaks. This suggests that the quick-soak method prefers a higher concentration of heavy-atom solutions.

### 3.2. Test case 2: derivatives of Fc$\gamma$RIII crystals

Fc$\gamma$RIII was crystallized in space group $P2_12_12$ and diffracted to 1.8 Å. The structure of Fc$\gamma$RIII has been determined (Zhang *et al.*, 2000). Both trimethyllead acetate (TMLA) and $HgCl_2$ were shown to derivatize the receptor (Sun & Hammer, 2000). Diffraction data were collected from three TMLA derivatization soaks: (5 m*M*, 10 min), (10 m*M*, 10 min) and (10 m*M*, 24 h). Similar to the lysozyme tests, the (10 m*M*, 10 min) soak resulted in better heavy-atom derivatization than the 24 h soak (Table 2, Figs. 3*a*–3*c*). A comparison between the two 10 min soaks with 5 and 10 m*M* TMLA, respectively, showed that the heavy-atom occupancies in the (10 m*M*, 10 min) soak are more than twice those in the (5 m*M*, 10 min) soak, suggesting that a higher concentration of the heavy-atom solution produces more complete derivatization.

The solubility of $HgCl_2$ is estimated to be less than 5 m*M* in the Fc receptor crystallization buffer. All overnight soaks lead to overall lattice disorder. However, diffraction data were collected from a 10 min and a 2 h soaked crystal (Table 2). The highest occupancy Hg-binding site (Site 1) produced 7.6 and 24.4$\sigma$ peaks in the difference Fourier maps from the 10 min and 2 h soaks, respectively (Figs. 3*d* and 3*e*). The higher occupancies from the 2 h soak than from the 10 min soak indicate that $HgCl_2$ reacts more slowly than TMLA and that the optimal length of time for soaking reactions may vary depending on the heavy-atom compound. In principle, it should be possible to estimate the optimal soaking time using a solution reaction since the rate of a chemical reaction in solution is correlated with that in the crystalline state. As an

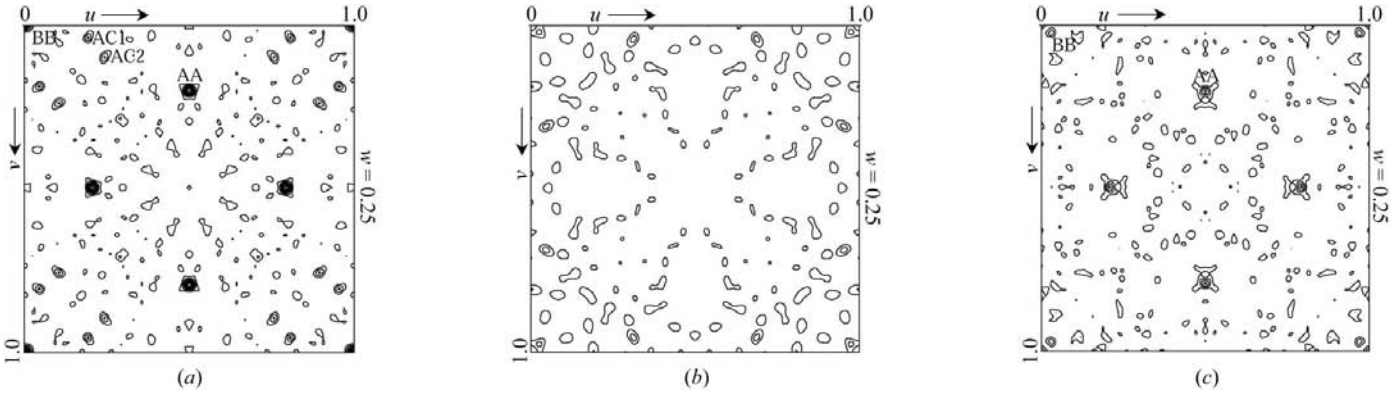


**Figure 2**
Difference Patterson map of the lysozyme $KAuCl_4$ derivative at the Harker section $w = 0.25$. All maps are contoured at the 1$\sigma$ level with a 1$\sigma$ increment. The $KAuCl_4$ soaking conditions are (*a*) (10 m*M*, 10 min), (*b*) (10 m*M*, 24 h) and (*c*) (1 m*M*, 48 h). The peak AA is a Harker peak of site 1 at $w = 0.25$. Peak BB is a non-Harker self-Patterson peak of site 2. Peaks AC1 and AC2 are two cross Patterson peaks between sites 1 and 3.

example, the reaction kinetics of $HgCl_2$ derivatization of Fc$\gamma$RIII was monitored in solution using mass spectrometry. The result showed that larger amounts of both the one- and two-$Hg^{2+}$ bound Fc$\gamma$RIII formed from a 2 h reaction than from a 10 min reaction (Fig. 4).

### 3.3. Comparison with conventional soaking

The quick-soak method offers three main advantages over the conventional heavy-atom soaking method. Firstly, it preserves the diffraction resolution. In all data tested, the quick soak resulted in no obvious deterioration in diffraction resolution compared with that from a native crystal. In contrast, data collected from overnight-soaked crystals often showed a reduction in both the resolution and data quality. In some cases, the longer overnight soaks resulted in complete lattice disorder. The success of a quick rather than a prolonged soak suggests that there are two kinetically distinct processes occurring during derivatization: a rapid specific metal-binding reaction and a slower lattice-disordering process. They are not distinguished in conventional lengthy soaks, but can be separated in quick soaks, which prevent the latter from progressing to a level that will affect data quality and isomorphism. This kinetic separation is likely to be even more noticeable for non-covalent electrostatically bound heavy-atom compounds, in which the rate of derivatization is limited by the rate of diffusion. For covalent reactions, the rate of heavy-atom reactions varies depending on the solution pH, buffer and the presence of other groups. This will undoubtedly affect the soaking time in quick-soak experiments. For example, the optimal soaking time for the $HgCl_2$ derivative of Fc$\gamma$RIII was 2 h rather than 10 min. Secondly, the quick-soak method minimizes the non-isomorphism associated with a derivative data set. This is reflected by a smaller observed change in unit-cell parameters from the native in all quick-soak data sets compared with data sets from 24 h or longer soaking (data not shown). In the lysozyme case, in which neither short nor long soaking caused significant changes in unit-cell parameters, the effect of non-isomorphism is still less in short soaks as evident from a generally lower $R_{iso}$ but higher heavy-metal phasing occupancy. Finally, the quick-soak method saves time. The current heavy-atom derivatization method was developed in earlier years of protein crystallography when data collections were performed at room temperature and typically took several days to complete. Quick soaking would in fact make things worse under those conditions because the slower crystal lattice transformation process would not have completed when data collection started. This would result in the earlier data frames being inconsistent with the later frames. There-

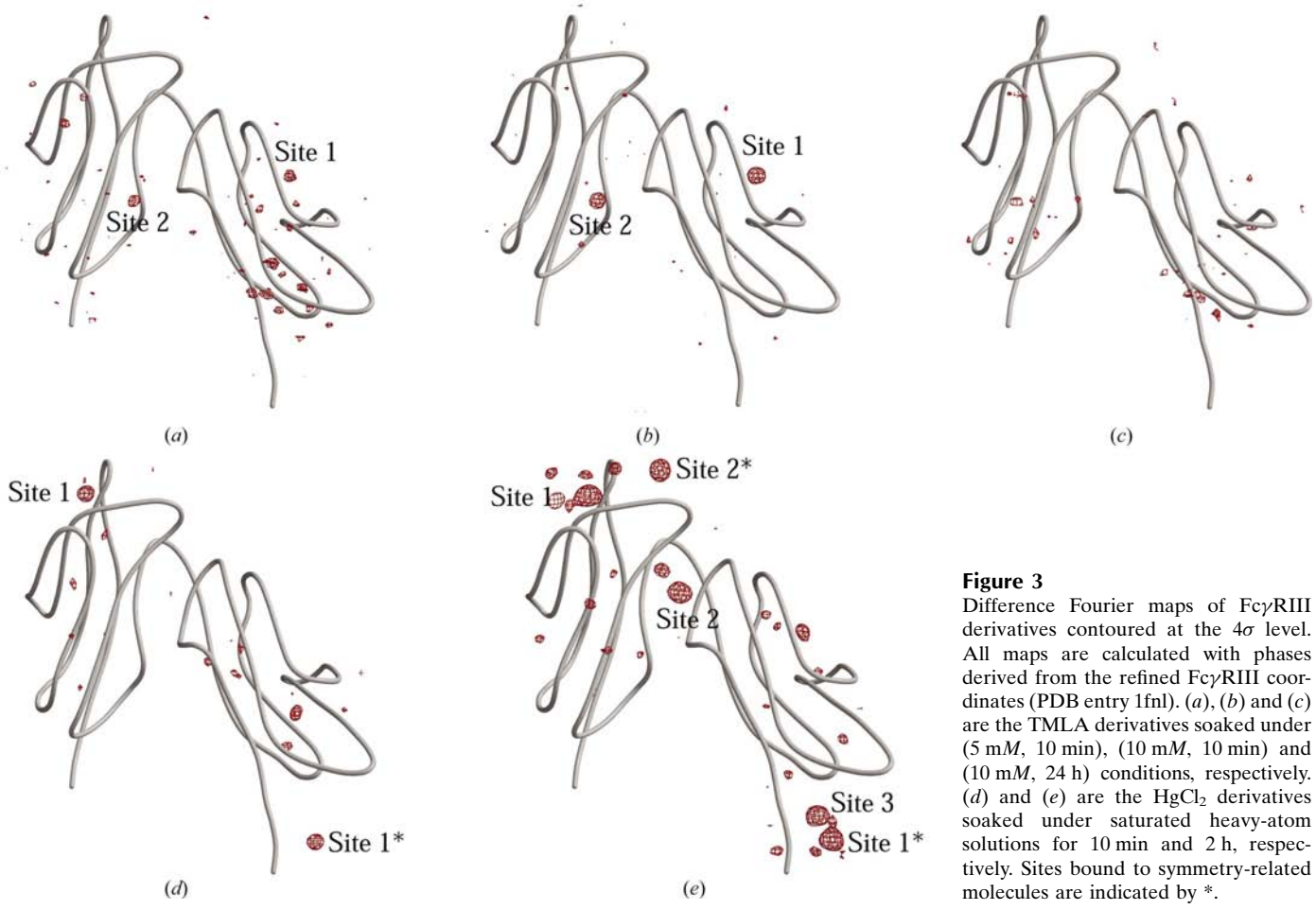


**Figure 3**
Difference Fourier maps of Fc$\gamma$RIII derivatives contoured at the 4$\sigma$ level. All maps are calculated with phases derived from the refined Fc$\gamma$RIII coordinates (PDB entry 1fnl). (*a*), (*b*) and (*c*) are the TMLA derivatives soaked under (5 m*M*, 10 min), (10 m*M*, 10 min) and (10 m*M*, 24 h) conditions, respectively. (*d*) and (*e*) are the $HgCl_2$ derivatives soaked under saturated heavy-atom solutions for 10 min and 2 h, respectively. Sites bound to symmetry-related molecules are indicated by *.

fore, it would be better to start data collections after crystal lattice change was complete. The improvement in detector technology in recent years has speeded up crystallographic data collection substantially. More importantly, the application of low temperature in routine data collection has made it possible to trap a heavy-atom reaction and freeze the lattice-transformation process. Combined with rapid synchrotron data collection, the quick-soak method offers a potential 'on-the-fly' and real-time heavy-atom screening that is particularly important in the age of structural genomics.

### 3.4. Choice of heavy-atom concentration and soaking time

For conventional overnight soaks, the concentration of a heavy-atom reagent is often limited by the adverse effect of the heavy-atom reagent on the crystal lattice and diffraction resolution. This adverse effect is negligible in all quick soaks tested for lysozyme and Fc$\gamma$RIII crystals. Consequently, a higher concentration of heavy-atom reagents should be used in quick soaks for the benefit of thorough derivatizations, as the rate of a chemical reaction is proportional to the concentration of heavy-atom reagents. In both lysozyme and Fc$\gamma$RIII tests, the highest heavy-atom occupancies were obtained with 10 m*M* or higher concentrations of the heavy-atom reagents. Quick soaks with varying heavy-atom concentrations showed that less than optimal derivatizations were obtained with heavy-atom concentrations below 10 m*M*. In practice, it may be beneficial to use saturated heavy-atom solutions for quick-soak screening experiments, as many of the heavy-atom compounds have only limited solubility in crystallization solutions.

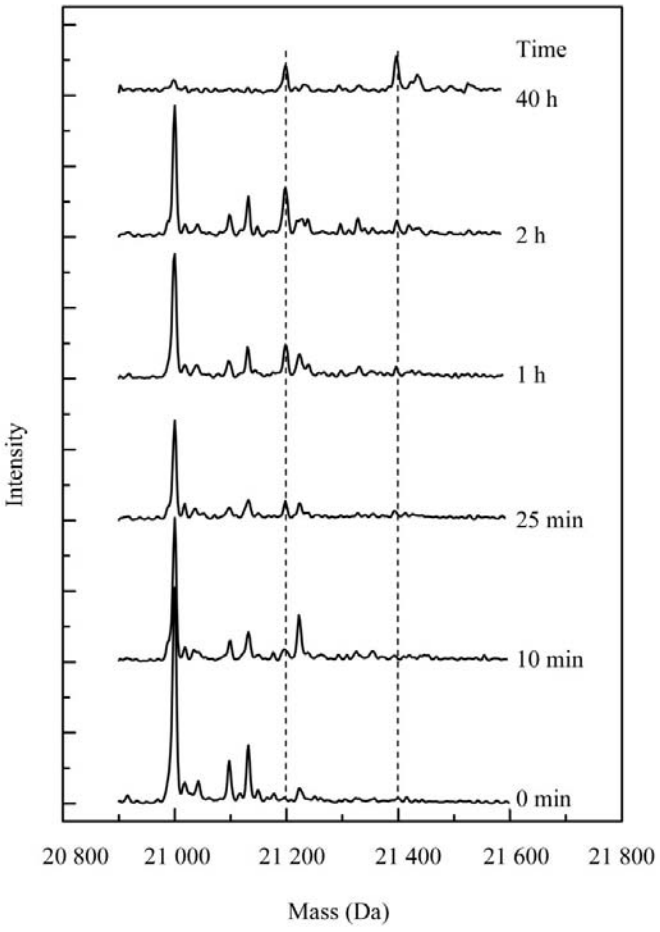


**Figure 4**
Reaction kinetics of $HgCl_2$ derivatization of Fc$\gamma$RIII as monitored by mass-spectrometry measurements. Approximately the same amount of sample was analyzed at each time point. Shown here are the derivatization reactions at $t$ = 0, 10 and 25 min, 1, 2 and 40 h time points. The curves are displaced along the vertical axis for clarity. The native Fc$\gamma$RIII has a mass of 21 000 Da. The one- and two-$Hg^{2+}$ bound receptors, whose peak positions are indicated by vertical dashed lines, display masses of 21 000, 21 198 and 21 396 Da, respectively. The 40 h reaction sample displayed a significant amount of general aggregate (data not shown) compared with other time points. This presumably is a consequence of the binding of multiple non-specific $Hg^{2+}$ to the receptor and thus contributed to the overall loss in mass-spectrometry signals for the native and one-$Hg^{2+}$ bound receptor forms.

Most of the quick-soak experiments were carried out between 10 min and 2 h. Longer soaking time favors a complete derivatization reaction but increases the non-isomorphism with respect to the native as well as the lattice disorder. The observation that a 2 d soaked lysozyme crystal yielded smaller heavy-atom peaks than a 10 min soaked one suggests that a complete heavy-atom reaction can be achieved in 10 min and that the smaller peaks from the longer soak are likely to be the result of crystal non-isomorphism. Shorter than 10 min soaks are not necessary since 10 min soaks produced no significant crystal non-isomorphism. The optimum soaking length should represent the balance between the desires to achieve the highest heavy-atom binding occupancy and to minimize soak-related crystal non-isomorphism.

Quick soaking should be particularly useful in situations where prolonged soaking leads to either crystal lattice disorder or large non-isomorphism as reflected in large values of $R_{\rm iso}$ but with no interpretable heavy-atom binding sites. For these derivatives, shortening the soaking time rather than reducing the heavy-atom concentration should effectively minimize soaking-induced lattice transformations and may thus lead to generating useful derivatives.

### 3.5. Comparison with the halide-soaking method

Recently, a halide-soaking method was proposed as an alternative way of phasing protein diffraction data (Dauter *et al.*, 2000). The method is based on the ability of heavier halide ions, such as $Br^-$ and $I^-$, to replace smaller $Cl^-$ ions bound at protein surfaces. To achieve an effective ion replacement, the concentration of $Br^-$ and $I^-$ salts is recommended to be between 0.5 and 1 *M*. Since halide ions are electrostatically bound to protein surfaces and the rate of derivatization is only limited by the rate of diffusion, and since many crystal lattices are not stable under such concentrated salt conditions, the soaking is limited to 1 min or less. Owing to the intrinsic low binding affinity, the average halide occupancy was found to be less than 50%. This halide-soaking method was later extended to include other electrostatically bound ions such as $Cs^+$, $Rb^+$, $Gd^{3+}$ and $Sm^{3+}$ (Nagem *et al.*, 2001). The current quick-soak

method is not an extension of the halide-soaking method. It is, in fact, quite different from the halide-soaking method and is more appropriately viewed as an improvement of the conventional heavy-atom soaking method. Firstly, the mechanism of heavy-atom attachment to protein can be either covalent or electrostatic and generally involves unique sites that are different from halide-binding sites. Secondly, the required reaction time is between 10 min and a few hours. It is generally longer than 1 min owing to the lower concentrations used and the fact that a chemical reaction rate is slower than the diffusion rate. Thirdly, the concentration of heavy atom is generally between 10 and 50 m*M*, similar to conventional heavy-atom solutions but much less than the concentration of halide ions. Under these mild concentrations, crystal lattices are generally more stable, so that a soaking of a few hours often does not result in extensive lattice disorder.

In conclusion, we have demonstrated the validity of using a quick-soak method to generate heavy-atom derivatives for protein phasing. The essence of this method is to derivatize proteins with a near-saturation concentration of heavy-atom compounds in a short time. Specifically, we recommend (i) using heavy-atom solution concentrations between 10 m*M* and saturation for initial derivatization reactions and (ii) varying crystal soaking times from 10 min to a few hours to maximize heavy-atom occupancies.

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