Direct observation of bond formation in solution with femtosecond X-ray scattering

Kyung Hwan Kim^{1,2}*, Jong Goo Kim^{1,2}*, Shunsuke Nozawa³*, Tokushi Sato³†*, Key Young Oang^{1,2}, Tae Wu Kim^{1,2}, Hosung Ki^{1,2}, Junbeom Jo^{1,2}, Sungjun Park^{1,2}, Changyong Song⁴, Takahiro Sato⁴†, Kanade Ogawa⁴†, Tadashi Togashi⁵, Kensuke Tono⁵, Makina Yabashi⁴, Tetsuya Ishikawa⁴, Joonghan Kim⁶, Ryong Ryoo^{1,2}, Jeongho Kim⁷, Hyotcherl Ihee^{1,2} & Shin-ichi Adachi^{3,8}

The making and breaking of atomic bonds are essential processes in chemical reactions. Although the ultrafast dynamics of bond breaking have been studied intensively using time-resolved techniques¹⁻³, it is very difficult to study the structural dynamics of bond making, mainly because of its bimolecular nature. It is especially difficult to initiate and follow diffusion-limited bond formation in solution with ultrahigh time resolution. Here we use femtosecond time-resolved X-ray solution scattering to visualize the formation of a gold trimer complex, $[Au(CN)_2]_3$ in real time without the limitation imposed by slow diffusion. This photoexcited gold trimer, which has weakly bound gold atoms in the ground state⁴⁻⁶, undergoes a sequence of structural changes, and our experiments probe the dynamics of individual reaction steps, including covalent bond formation, the bentto-linear transition, bond contraction and tetramer formation with a time resolution of ~500 femtoseconds. We also determined the three-dimensional structures of reaction intermediates with subångström spatial resolution. This work demonstrates that it is possible to track in detail and in real time the structural changes that occur during a chemical reaction in solution using X-ray free-electron lasers⁷ and advanced analysis of time-resolved solution scattering data.

The functional efficiencies of photoactive molecules are governed by long-lived electronic excited states that are directly involved in functional transitions of the molecules. In fact, early stages of their photoinduced reactions, involving bond breaking and bond making, determine the fate of the excited molecules and it is therefore crucial to understand the mechanism of the initial reaction steps leading to the functional transitions. For several decades, ultrafast bond-breaking processes in various molecular systems have been studied intensively using time-resolved techniques¹⁻³. Unlike bond breaking, which is essentially a unimolecular process and can therefore be initiated by laser photolysis in a synchronized manner, bond making is in most cases a bimolecular process that requires two reactant parties to meet each other to form a chemical bond. The reaction rate of a bimolecular process is generally limited by slow diffusion of the reactants through the solvent and it is thus difficult to synchronize laser excitation with the moment that they meet. Therefore, it is challenging to initiate and follow the bimolecular process with ultrahigh time resolution, although a few special experimental schemes have overcome this obstacle in the case of electron or proton transfer reactions8,9.

In this regard, a Au oligomer complex, $[Au(CN)_2]_n$ offers a good model system in which to study the dynamics of bond formation in solution^{4,10-12}. Au(I) atoms in $[Au(CN)_2]_n$ experience a non-covalent interatomic interaction caused by the relativistic effect called aurophilicity^{11,12}. Owing to aurophilicity, Au(I) atoms can be weakly bound to each other by van der Waals interactions, forming an aggregate complex

 $[Au(CN)_2^-]_n$ even without covalent bonds. On photoexcitation of the complex, an electron is excited from an antibonding orbital to a bonding orbital, leading to the formation of covalent bonds among Au atoms⁴. Because Au atoms in the ground state of $[Au(CN)_2^-]_n$ are located in close proximity within the same solvent cage, the formation of covalent Au–Au bonds occurs without being limited by slow diffusion through the solvent. Therefore, the ultrahigh time resolution necessary to probe this bond-making process can be achieved, as in typical unimolecular reactions synchronized with laser photolysis, but the ensuing reaction is like a bimolecular reaction between $Au(CN)_2^-$ monomers.

Recently, ultrafast Au-Au bond formation in a Au trimer complex, $[Au(CN)_2]_3$, was investigated using transient absorption spectroscopy⁵, and the transient changes in absorption were observed with time constants of variously 500 fs, 2 ps and 2 ns. The first kinetic component (500 fs) was ascribed to the intersystem crossing to a triplet state, which is presumably preceded by rapid contraction of Au-Au bonds within 500 fs. The second kinetic component (2 ps) was assigned to conformational change from bent to linear structure. However, because the transient absorption signal is not directly related to the molecular structure, those structural assignments were based solely on theoretical electronic absorption spectra of model structures. As a result, those structural assignments were disputed by a recent study using quantum chemical calculation⁶. According to the *ab initio* molecular dynamics simulation, the bent-to-linear transition occurs on the 500 fs timescale but was assigned to the 2 ps kinetic component in the transient absorption study. Such discrepancies between experiment and theory, mainly due to limited structural information obtained from experiments, are not limited to this particular system but are a common problem in chemistry in general.

To resolve this discrepancy, we applied time-resolved X-ray solution scattering13 (TRXSS) to the same Au trimer complex. TRXSS is an effective method for probing photoinduced structural changes of molecules in solution and has been used to reveal the dynamics and mechanism of various molecular reaction systems ranging from small molecules14-17 to biological macromolecules^{18,19}. Although subpicosecond time resolution has been achieved in the case of X-ray²⁰⁻²³ and electron²⁴⁻²⁶ diffraction of solid samples and in X-ray absorption spectroscopy²⁷ of liquid samples, the temporal resolution of TRXSS based on synchrotron radiation¹³ has been limited to only 100 ps, thus preventing the observation of ultrafast processes on the timescales of femtoseconds to picoseconds. This limit can be overcome with the recent development of X-ray free-electron lasers (XFELs), which generate ultrashort (<100 fs long) X-ray pulses with $\sim 10^{12}$ photons per pulse^{28,29}. As a result, it has become possible to explore chemical and biological processes occurring on subpicosecond timescales using TRXSS³⁰. In this work, by performing

¹Center for Nanomaterials and Chemical Reactions, Institute for Basic Science, Daejeon 305-701, South Korea. ²Department of Chemistry, KAIST, Daejeon 305-701, South Korea. ³Institute of Materials Structure Science, High Energy Accelerator Research Organization, 1-1 Oho, Tsukuba, Ibaraki 305-0801, Japan. ⁴RIKEN SPring-8 Center, Kouto 1-1-1, Sayo, Hyogo 679-5148, Japan. ⁵Japan Synchrotron Radiation Research Institute, Kouto 1-1-1, Sayo, Hyogo 679-5198, Japan. ⁵Department of Chemistry, The Catholic University of Korea, Bucheon 420-743, South Korea. ⁷Department of Chemistry, Inha University, Incheon 402-751, South Korea. ⁸Department of Materials Structure Science, School of High Energy Accelerator Science, The Graduate University for Advanced Studies, 1-1 Oho, Tsukuba, Ibaraki 305-0801, Japan. ⁴Present addresses: Center for Free-Electron Laser Science, Deutsches Elektronen-Synchrotron, Notkestrasse 85, 22607 Hamburg, Germany (Tokushi Sato); Department of Chemistry, School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan (Takahiro Sato); Japan Atomic Energy Agency, 8-1-7 Umemidai, Kizugawa, Kyoto 619-0215, Japan (K.O.). *These authors contributed equally to this work.

RESEARCH LETTER



Figure 1 | Femtosecond time-resolved X-ray solution scattering at the XFEL facility and the data analysis. a, The photochemical reaction of solutes supplied by a liquid-flowing system is triggered by a femtosecond optical laser pulse. Subsequently, a time-delayed X-ray pulse synchronized with the laser pulse probes the structural dynamics of the reaction. The scattering pattern is detected by a fast two-dimensional charge-coupled device (CCD) detector as shown at the bottom. We measure time-resolved scattering patterns while varying the time delay between the laser and X-ray pulses. **b**, By integrating the

the TRXSS experiment at an XFEL facility⁷ (SACLA), we were able to study the ultrafast structural dynamics of bond formation in $[Au(CN)_2^-]_3$ in solution with subpicosecond time resolution and sub-ångström spatial resolution.

The femtosecond TRXSS experiment performed in this study is shown schematically in Fig. 1, and the details of the experimental procedure and the analysis are described in Methods and Extended Data Table 1. two-dimensional scattering pattern azimuthally, subtracting solvent contributions, performing a Fourier transform (FT) and compensating for the depletion of the initial solute contribution due to photochemical reaction, we obtain one-dimensional RDFs in real space as shown in the plot at the top left. These display the interatomic distances of transient species and products. In this way, Au–Au bond lengths of the $[Au(CN)_2^-]_3$ complex can be identified with sub-ångström accuracy, and the time-dependent structural changes of the metal complex can be determined in real time.

For the sample, we used an aqueous solution of $Au(CN)_2^-$ at a concentration of 300 mM, because in a solution of this concentration the $[Au(CN)_2^-]_3$ trimer has much higher absorbance at 267 nm than does a monomer or a dimer of $Au(CN)_2^-$ (Supplementary Information). As a result, the laser excitation at 267 nm used in our TRXSS experiment predominantly excites the $[Au(CN)_2^-]_3$ trimer while exciting the other species negligibly. From the TRXSS experiment, we obtained difference





a, Experimental difference scattering curves, $q\Delta S(q)$, measured at various time delays from -800 fs to 300 ns (black). For clarity, only data at selected time delays are shown. a.u., arbitrary units. **b**, RDFs, $r^2S(r)$, obtained by Fourier sine transformation of $q\Delta S(q)$ after subtracting solvent contributions. The RDF of the S₀ state was added to the RDFs at all time delays to emphasize only the contributions of the transient solute species associated with bond formation. The blue dashed arrows indicate the time-dependent changes in the locations of the p₁ and p₂ peaks. The red dashed line represents the position of the p₃ peak, corresponding to the signature of the transiton kinetics. The notation for each species is indicated above each trace. The error bar at each data point

indicates the standard error determined from 50 independent measurements (that is, 50 scattering images). The vertical black dotted lines indicate the temporal positions corresponding to the time constants of the three kinetic components. **d**, Species-associated RDFs of the four structures obtained from the singular value decomposition and principal-component analyses (black) and their fits (red) obtained by using model structures containing multiple Au–Au pairs. The blue arrows indicate the changes in R_{12} , R_{23} and R_{13} as transitions occur between states. As fitting parameters, we considered three Au–Au pairs for the S_0 , S_1 and T_1 states and six Au–Au pairs for the tetramer. For each state, the structural parameters obtained from the fits are shown along with their standard errors determined from 50 independent measurements.





Figure 3 Structure determination of the S₁ state using the experimental scattering curve at 200 fs time delay in momentum space and real space. a, Theoretical difference scattering curves (red) for linear (upper) and bent (lower) structures shown together with the experimental difference scattering curve at 200 fs (black). The residuals (blue) between the theoretical and the experimental curves are shown together. The linear structure gives a much

better fit than the bent structure, which has the same Au–Au–Au bond angle as the S_0 state, thereby indicating that the bent-to-linear transition is completed at 200 fs time delay. **b**, Corresponding experimental (black) and theoretical (red) RDFs, rS(r). It can be seen that in the bent structure R_{13} is too small to fit the experimental RDF at 200 fs.

scattering curves, $q\Delta S(q, t)$, where q is the momentum transfer between the incident and the elastically scattered X-ray waves and ΔS is the difference between scattering intensities measured before the laser excitation (that is, at a negative time delay) and after the laser excitation (that is, at a positive time delay). The experimental difference scattering curves measured at various time delays from -800 fs to 300 ns are shown in Fig. 2a. The difference scattering curves show distinct oscillatory features along the *q* axis, which indicate large structural changes in $[Au(CN)_2^-]_3$ during the formation of covalent Au–Au bonds. Considering that the oscillatory features appear distinct even after the shortest time delay



Figure 4 | Mechanism of photoinduced bond formation in $[Au(CN)_2]_3$. a, Femtosecond TRXSS reveals the dynamics and the atomic movements associated with the Au–Au bond formation in real time with sub-ångström spatial resolution. The S₀ state with weakly bound Au atoms in a bent geometry transforms to the S₁ state with tightly bound Au atoms in a linear geometry. Subsequently, the S_1 state transforms first to the T_1 state, with further contraction of Au–Au bonds, and then to a tetramer through formation of another Au–Au bond. **b**, Structural parameters of each state and their standard errors determined from 50 independent measurements.

(200 fs), the first step of bond formation in $[Au(CN)_2^-]_3$ must occur impulsively within the time resolution (~500 fs). The oscillatory features change further over time until only the oscillatory contribution from solvent heating remains after 300 ns (Extended Data Fig. 2).

A more intuitive picture of the structural change in $[Au(CN)_2^{-}]_3$ can be obtained when we convert $q\Delta S(q, t)$ into difference radial distribution functions (RDFs), $r^2\Delta S(r, t)$, in real space by Fourier sine transformation. To emphasize only the contributions of transient solute species associated with bond formation, we added the RDF of the ground (S₀) state to the difference RDFs at all time delays and obtained the RDFs $r^2S(r,t)$ shown in Fig. 2b. Because the solvent contributions were eliminated and the contributions from C and N atoms in $[Au(CN)_2^{-}]_3$ are almost negligible compared with the strong scattering from Au atoms (Supplementary Information and Extended Data Fig. 6), the RDFs shown in Fig. 2b actually represent the interatomic distances among Au atoms of $[Au(CN)_2^{-}]_3$ in real space.

It can be seen that two peaks (p1 and p2) are distinct in the RDFs in Fig. 2b. Because $[Au(CN)_2]_3$ is a trimer, we can assign the p₁ peak to the Au₁-Au₂ pair (bond length, R_{12}) and the Au₂-Au₃ pair (bond length, R_{23}), and the p₂ peak to the Au₁-Au₃ pair (bond length, R_{13}). This assignment is supported by the observation that the intensity of p_1 is about twice as large as that of p_2 . In the S_0 state of $[Au(CN)_2]_3$ (that is, RDF at -800 fs time delay), R_{12} and R_{23} (~3.6 Å as indicated by the position of p₁) are relatively large compared with the typical length of an Au–Au covalent bond (\sim 2.7 Å), indicating that the Au atoms are weakly bound, and R_{13} (~5.56 Å as indicated by the position of p_2) is smaller than the sum of R_{12} and R_{23} , indicating that the S₀ state has a bent structure. As expected in the momentum-space data, the RDF at 200 fs time delay is significantly different from the one at -800 fs, suggesting that the first step of bond formation in $[Au(CN)_2]_3$ occurs within the time resolution of our experiment. Compared with the S₀ state, at 200 fs time delay R₁₃ has increased slightly and R₁₂ and R₂₃ have decreased significantly, indicating the formation of covalent Au-Au bonds at this step. Simultaneously, R_{13} (~5.63 Å) becomes similar to the sum of R_{12} and R_{23} (~2.8 Å each), which is evidence of a conformational transition from bent to linear geometry. We note that the timescale of the bent-to-linear transition determined from our TRXSS experiment is in good agreement with the timescale predicted from the previous theoretical study⁶. From 200 fs to 10 ps, the p_1 and p_2 peaks shift to smaller distances, indicating further decrease of the Au-Au distances due to the formation of stronger covalent Au-Au bonds. In this time range, the ratio between $R_{12} + R_{23}$ and R_{13} remains 1:1, showing that the linear structure is preserved. After 100 ps, the p_3 peak appears at ~8.5 Å and increases until a time delay of 10 ns. Because the position of the p₃ peak corresponds to too great a Au-Au distance for the complex to be a trimer, p₃ must be a signature of the formation of a tetramer complex. Also, increased intensities of the p1 and p2 peaks imply the presence of a larger number of Au-Au pairs in the tetramer. After 10 ns, the RDF returns gradually to the RDF of the S₀ state.

By singular value decomposition (SVD) and principal-component analysis, we obtained species-associated RDFs for four states, the ground (S_0) state, an excited (S_1) state, a triplet (T_1) state and a tetramer (Fig. 2d), as well as their kinetics. We fitted the experimental RDFs at various time delays by linear combinations of the species-associated RDFs, and determined the time-dependent concentration of each state from the coefficient of the corresponding species-associated RDF (Fig. 2c). As a result, we obtained three kinetic components with time constants of 1.6 ± 0.1 ps, 3 ± 0.5 ns and 100 ± 20 ns, which correspond respectively to the transition from S₁ to T₁, the transition from T₁ to the tetramer, and the transition from the tetramer to S_0 . The timescales of the three kinetic components match well with the ones identified in the previous transient absorption study⁵, except our TRXSS data lack the \sim 500 fs component, which in the transient absorption study was assigned to intersystem crossing to a triplet state. Here we note that TRXSS is sensitive only to processes accompanying structural changes. Therefore, the fact that the \sim 500 fs kinetic component is not observed by TRXSS

indicates that intersystem crossing does not involve any significant structural change (see Extended Data Fig. 9 for a detailed kinetic scheme).

To reconstruct the structures of the four states $(S_0, S_1, T_1 \text{ and tetramer})$ and extract the Au-Au distances for each state, we performed a structural fitting analysis for the species-associated RDF of each state using the Au-Au distances as fitting parameters. The fitting results for the four states are shown in Fig. 2d. Reconstructed structures based on the optimized Au-Au distances are shown together. The reconstructed structures of the four states presented in Fig. 2d are in good agreement with the structural changes inferred from Fig. 2b. The S₀ state has weakly bound Au atoms in a bent geometry with R_{12} and R_{23} different from each other, which difference can be attributed to a broadening of the RDF induced by relatively free movements of the weakly bound Au atoms (Supplementary Information and Extended Data Fig. 10). The S₁ state has much shorter Au–Au distances than does the S₀ state, owing to the formation of covalent Au-Au bonds. Notably, the S1 state has Au atoms aligned in a linear geometry, confirming that the bent-to-linear transition occurs during the transition to the S₁ state. We also find that the two Au–Au bonds in the S₁ state are the same length, indicating the symmetric structure of S1. The T1 state has even shorter Au-Au distances than does S₁, owing to the formation of stronger covalent Au–Au bonds, and retains a linear and symmetric structure. Finally, we clearly identified the structure of the tetramer, $[Au(CN)_2]_4$, as the final species formed before ultimately returning to the S₀ state. The changes in the Au–Au distances and the conformations of the S₀, S₁, T₁ and tetramer states in Fig. 2d are in good agreement with the results of the previous theoretical calculation⁶.

The previous transient absorption study identified the changes of transient absorption on 500 fs, 2 ps and 2 ns timescales⁵. The timescales of the transitions found in the transient absorption study match well the results of this work, but the detailed structural changes were assigned differently. In particular, from our TRXSS measurement we inferred that the bent-to-linear transition occurs within a few hundred femtoseconds rather than on the timescale of 2 ps. To account for this discrepancy, we determined the structure of the S₁ state more carefully by fitting the experimental difference scattering curve at 200 fs time delay using two different model structures (Fig. 3). One is a linear structure where R_{13} is equal to the sum of R_{12} and R_{23} , and the other is a bent structure where the Au-Au-Au bond angle of the S1 state is the same as that of the S₀ state. In Fig. 3a, it is clearly seen that the theoretical scattering curve calculated from the linear structure ($\chi^2 = 1.70$) fits the experimental scattering curve at 200 fs time delay much better than does the one calculated from the bent structure ($\chi^2 = 4.81$), where χ^2 is a quantitative measure of the discrepancy between the experimental and theoretical scattering curves (Methods). The difference between the linear and bent structures can be seen more distinctly in the real-space RDFs in Fig. 3b. In the bent structure, R_{13} is too small to fit the experimental RDF at 200 fs. Therefore, the bent-to-linear transition must be completed at 200 fs time delay.

On the basis of the reconstructed structures of the four different states and the transition dynamics among them, in Fig. 4 we summarize the mechanism for photoinduced formation of Au–Au covalent bonds in $[Au(CN)_2^-]_3$. The S₀ state with weakly bound Au atoms in a bent geometry transforms within a few hundred femtoseconds to the S₁ state with tightly bound Au atoms (2.8 Å Au–Au distances) in a linear and symmetric geometry. The S₁ state transforms to the T₁ state with a 1.6 ps time constant accompanying further contraction of the Au–Au bonds by 0.1 Å. Then the T₁ state converts to a tetramer in 3 ns through formation of another Au–Au bond, and the S₀ state is ultimately recovered in ~100 ns.

We have demonstrated the capability of XFEL-based femtosecond TRXSS by both determining the overall mechanism for the formation of covalent Au–Au bonds in the $[Au(CN)_2]_3$ complex and gaining rich structural information. Femtosecond TRXSS offers a means of visualizing the entire process of photoinduced reactions in real time and real space, and can be used as a fundamental tool to study the reaction dynamics of chemical and biological systems.



Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 5 September; accepted 19 December 2014.

- 1. Zewail, A. H. Laser femtochemistry. Science 242, 1645–1653 (1988).
- Harris, A. L., Brown, J. K. & Harris, C. B. The nature of simple photodissociation reactions in liquids on ultrafast time scales. *Annu. Rev. Phys. Chem.* **39**, 341–366 (1988)
- Jonas, D. M., Bradforth, S. E., Passino, S. A. & Fleming, G. R. Ferntosecond wavepacket spectroscopy: influence of temperature, wavelength, and pulse duration. J. Phys. Chem. 99, 2594–2608 (1995).
- Rawashdeh-Omary, M. A., Omary, M. A., Patterson, H. H. & Fackler, J. P. Excitedstate interactions for [Au(CN)₂⁻]_n and [Ag(CN)₂⁻]_n oligomers in solution. Formation of luminescent gold-gold bonded excimers and exciplexes. *J. Am. Chem. Soc.* **123**, 11237–11247 (2001).
- Iwamura, M., Nozaki, K., Takeuchi, S. & Tahara, T. Real-time observation of tight Au–Au bond formation and relevant coherent motion upon photoexcitation of [Au(CN)₂⁻] oligomers. *J. Am. Chem. Soc.* **135**, 538–541 (2013).
 Cui, G. L., Cao, X. Y., Fang, W. H., Dolg, M. & Thiel, W. Photoinduced gold(I)-gold(I)
- Čui, G. L., Čao, X. Y., Fang, W. H., Dolg, M. & Thiel, W. Photoinduced gold(I)-gold(I chemical bonding in dicyanoaurate oligomers. *Angew. Chem. Int. Ed.* 52, 10281–10285 (2013).
- Tamasaku, K. *et al.* X-ray two-photon absorption competing against single and sequential multiphoton processes. *Nature Photon.* 8, 313–316 (2014).
- Rini, M., Magnes, B. Z., Pines, E. & Nibbering, E. T. J. Real-time observation of bimodal proton transfer in acid-base pairs in water. *Science* **301**, 349–352 (2003).
- Rosspeintner, A., Lang, B. & Vauthey, E. Ultrafast photochemistry in liquids. Annu. Rev. Phys. Chem. 64, 247–271 (2013).
 Pyykkö, P. Theoretical chemistry of gold. Angew. Chem. Int. Ed. 43, 4412–4456
- Pyykko, P. Theoretical chemistry of gold. Angew. Chem. Int. Ed. 43, 4412–4456 (2004).
- Wang, S. G. & Schwarz, W. H. E. Quasi-relativistic density functional study of aurophilic interactions. J. Am. Chem. Soc. 126, 1266–1276 (2004).
- Schmidbaur, H. & Schier, A. A briefing on aurophilicity. Chem. Soc. Rev. 37, 1931–1951 (2008).
- 13. Ihee, H. Visualizing solution-phase reaction dynamics with time-resolved X-ray liquidography. Acc. Chem. Res. **42**, 356–366 (2009).
- 14. Ihee, H. et al. ÚltrafastX-ray diffraction of transient molecular structures in solution. Science **309**, 1223–1227 (2005).
- Davidsson, J. et al. Structural determination of a transient isomer of CH₂I₂ by picosecond x-ray diffraction. Phys. Rev. Lett. 94, 245503 (2005).
- Production Array dimetation in Program (2000).
 Christensen, M. et al. Time-resolved X-ray scattering of an electronically excited state in solution. Structure of the ³A₂₄ state of tetrakis-µ-pyrophosphitodiplatinate(II). J. Am. Chem. Soc. **131**, 502–508 (2009).
- Kim, K. H. *et al.* Solvent-dependent molecular structure of ionic species directly measured by ultrafast X-ray solution scattering. *Phys. Rev. Lett.* **110**, 165505 (2013).
- Plech, A., Kotaidis, V., Lorenc, M. & Boneberg, J. Femtosecond laser near-field ablation from gold nanoparticles. *Nature Phys.* 2, 44–47 (2006).
- Kim, K. H. *et al.* Direct observation of cooperative protein structural dynamics of homodimeric hemoglobin from 100 ps to 10 ms with pump-probe X-ray solution scattering. J. Am. Chem. Soc. **134**, 7001–7008 (2012).

- Sokolowski-Tinten, K. et al. Femtosecond X-ray measurement of coherent lattice vibrations near the Lindemann stability limit. Nature 422, 287–289 (2003).
- Fritz, D. M. et al. Ultrafast bond softening in bismuth: mapping a solid's interatomic potential with X-rays. Science 315, 633–636 (2007).
- Coppens, P. Molecular excited-state structure by time-resolved pump-probe X-ray diffraction. What is new and what are the prospects for further progress? J. Phys. Chem. Lett. 2, 616–621 (2011).
- 23. Miller, T. A. *et al.* The mechanism of ultrafast structural switching in superionic copper(I) sulphide nanocrystals. *Nature Commun.* **4**, 1369 (2013).
- Zewail, A. H. Four-dimensional electron microscopy. Science 328, 187–193 (2010).
- Kirchner, F. O., Gliserin, A., Krausz, F. & Baum, P. Laser streaking of free electrons at 25 keV. Nature Photon. 8, 52–57 (2014).
- Miller, R. J. D. Mapping atomic motions with ultrabright electrons: the chemists' gedanken experiment enters the lab frame. *Annu. Rev. Phys. Chem.* 65, 583–604 (2014).
- Bressler, C. et al. Femtosecond XANES study of the light-induced spin crossover dynamics in an iron(II) complex. Science 323, 489–492 (2009).
- Lemke, H. T. *et al.* Femtosecond X-ray absorption spectroscopy at a hard X-ray free electron laser: application to spin crossover dynamics. *J. Phys. Chem. A* **117**, 735–740 (2013).
- 29. Zhang, W. et al. Tracking excited-state charge and spin dynamics in iron coordination complexes. *Nature* **509**, 345–348 (2014).
- Arnlund, D. et al. Visualizing a protein quake with time-resolved X-ray scattering at a free-electron laser. Nature Methods 11, 923–926 (2014).

Supplementary Information is available in the online version of the paper.

Acknowledgements We thank M. Iwamura and K. Nozaki for discussions. This work was supported by IBS-R004-G2; the X-ray Free Electron Laser Priority Strategic Program of MEXT, Japan; PRESTO/JST; the Innovative Areas 'Artificial Photosynthesis (AnApple)' (no. 25107527) grant from the Japan Society for the Promotion of Science; and the Basic Science Research Program through the National Research Foundation of Korea, funded by the Ministry of Science, ICT & Future Planning (NRF-2014R1A1A1002511). The experiments were performed at beamline BL3 of SACLA with the approval of the Japan Synchrotron Radiation Research Institute (proposal nos 2012A8030, 2012A8038, 2012B8029, 2012B8043, 2013A8053, 2013B8036, 2013B8059, 2014A8042 and 2014A8022) and at beamline NW14A of KEK with the approval of the Photon Factory Program Advisory Committee (proposal nos 2011G655, 2012G778 and 2012G779).

Author Contributions H.I. and S.-i.A. designed the study. K.H.K., J.G.K., S.N., Tokushi Sato, K.Y.O., T.W.K., H.K., J.J., S.P., C.S., Takahiro Sato, K.O., T.T., K.T., M.Y., T.I., Jeongho Kim, H.I. and S.-i.A. did the experiment. K.H.K., J.G.K., S.N., Tokushi Sato and Joonghan Kim analysed the data. K.H.K., J.G.K., S.N., K.Y.O., R.R., Jeongho Kim, H.I. and S.-i.A. wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to H.I. (hyotcherl.ihee@kaist.ac.kr) or S.-i.A. (shinichi.adachi@kek.jp).

METHODS

TRXSS data collection. The experimental set-up of femtosecond X-ray solution scattering is shown schematically in Fig. 1 in the main text. A laser pulse initiates a photoinduced reaction of the sample molecules and a time-delayed X-ray pulse probes the progress of the reaction. Time-resolved X-ray solution scattering data were collected at the BL3 beamline of SACLA and the NW14A beamline of KEK. X-ray pulses with sub-100 fs duration generated from SACLA were used for measuring the data at early time delays (from -800 fs to 100 ps), while X-ray pulses with 100 ps duration generated from KEK were used for measuring the data at early time delays (from -800 fs to 100 ps), while X-ray pulses with 100 ps duration generated from KEK were used for measuring the data at late time delays (from 100 ps to 1 µs). To check the reproducibility of the X-ray scattering signals at the two beamlines, we compared time-resolved difference scattering curves, $q\Delta S(q, t)$, measured at SACLA and KEK as shown in Extended Data Fig. 1a. The two difference scattering curves at a common time delay (100 ps) are identical to each other within the experimental error, indicating that our measurement is highly reproducible and independent of the facility. Difference scattering curves in the entire time range (from -800 fs to 1 µs) are shown in Extended Data Fig. 1b.

TRXSS data collection at SACLA. Time-resolved X-ray solution scattering measurements at early time delays (from -800 fs to 100 ps) were performed at the BL3 beamline of SACLA. Femtosecond laser pulses at 800 nm centre wavelength were generated from the Ti:sapphire regenerative amplifier and converted to 100 fs pulses at 267 nm wavelength by third-harmonic generation. The laser beam was focused by a lens to a spot of 300 μ m diameter, where the laser beam was overlapped with the X-ray beam with the crossing angle of 10°. The femtosecond X-ray pulses were generated from the XFEL at SACLA by self-amplified spontaneous emission (SASE). The X-ray pulses have a centre energy of 15 keV and an energy bandwidth narrow enough ($\Delta E/E = 0.6\%$) for monitoring photoinduced structural changes of small molecules^{31,32}. The X-ray beam was focused on a spot of 200 µm diameter at the sample position and the resultant X-ray fluence was 1.3 mJ mm⁻². The scattering patterns generated by X-ray pulses were measured with an area detector (Rayonix LX255-HS) with a sample-to-detector distance of 31 mm. We used aqueous solution of the oligomer of Au complex [Au(CN)2⁻]_n. The aggregation number of the Au oligomer and the position of an absorption peak in the ultraviolet region change depending on the concentration of the sample solution³³. In this work, we focus on the trimer, $[Au(CN)_2]_3$, that is formed in the solution of 300 mM concentration and can be excited by laser pulses of 267 nm centre wavelength. As shown in the Extended Data Fig. 7 and Supplementary Information, the signal is dominated by the contribution of the trimer while the contributions of other species are negligible. In fact, as shown in Extended Data Fig. 8, the TRXSS signals measured with the excitation at 267 and 310 nm, respectively, are identical to each other in terms of the kinetics and the shape of the difference scattering curves, confirming the dominant contribution of the trimer to the TRXSS signal. On photoexcitation, the trimer undergoes structural changes including variation in its geometric structure and the formation of covalent Au-Au bonds. The sample solution was circulated through a sapphire nozzle with a 100 µm-thick aperture. To supply fresh sample for every laser and X-ray shot, the sample-flowing velocity was set to be over 3 m s⁻¹ To prevent the scattering signal from being contaminated by radiation-damaged sample, the sample in the reservoir was replaced with fresh sample whenever the reservoir sample failed to produce the transient signal measured at 100 ps. Even if the transient signal at 100 ps did not change, the sample in the reservoir was replaced with fresh one regularly (every 2-3 h of measurement) to ensure the delivery of fresh sample. The structural change was monitored by the scattering patterns generated by X-ray pulses of sub-100 fs duration. The time resolution of the X-ray solution scattering experiment was \sim 500 fs, which was limited by the timing jitter between the laser and X-ray pulses as well as a velocity mismatch of 120 fs that was calculated by considering the laser/X-ray crossing angle of 10° and the sample thickness of 100 µm. The laser-off images were acquired with the X-ray pulse arriving 5 ps earlier than the laser pulse (that is, -5 ps time delay), to probe the (unexcited) molecules in the ground state while assuring the same average temperature of the sample solution. These laser-off images were repeatedly measured before every laser-on image and were used as a reference for calculating the time-resolved difference X-ray scattering patterns. To achieve a signal-to-noise ratio high enough for data analysis, about 50 images were acquired at each time delay. Each scattering image was obtained by accumulating scattering intensities of 80 X-ray pulses. The data collection scheme of accumulating multiple X-ray shots in a single scattering image was enough to effectively suppress the fluctuation of signal caused by random pulse energies of the SASE process at SACLA, as proven by the good agreement of two difference scattering curves measured at the XFEL and synchrotron radiation source (Extended Data Fig. 1a). The scattering curves were measured at the following time delays: -5 ps, -800 fs, -300 fs, 200 fs, 700 fs, 1.2 ps, 1.7 ps, 3.2 ps, 5.2 ps, 10 ps, 20 ps, 30 ps, 50 ps, 100 ps. The scattering signals arising from solvent (water) heating were also measured using 40 mM FeCl₃ solution with the same experimental conditions. Details of the data collection parameters are summarized in Supplementary Table 1.

TRXSS data collection at KEK. Time-resolved X-ray solution scattering measurement at late time delays (100 ps-1 µs) was performed at the NW14A beamline of KEK by following the experimental protocol described in our previous publications^{27,34,35}. Third-harmonic generation of the 800 nm output pulses from an amplified Ti:sapphire laser system provided 150 fs pulses at 267 nm centre wavelength. The laser beam was focused by a lens to a spot of 300 µm diameter, where the laser beam is overlapped with the X-ray beam with a crossing angle of 10°. The laser pulses were synchronized with X-ray pulses from the synchrotron using an active feedback control loop that adjusts the laser oscillator cavity length, and the relative time delay between the laser and X-ray pulses was controlled electronically. The timedelayed X-ray pulses were selected by using a synchronized mechanical chopper. A multilayer optic coated with depth-graded Ru/C layers (d = 40 Å, NTT Advanced Technology) produced a Gaussian-type X-ray spectrum with a centre energy of 15.6 keV and a \sim 5% energy bandwidth. The X-ray beam was focused on a spot of 200 µm diameter at the sample position, and the resultant X-ray fluence was $0.017 \text{ mJ} \text{ mm}^{-2}$. The scattering patterns generated by X-ray pulses of 100 ps (fullwidth at half-maximum) duration were measured with an area detector (MarCCD165, Mar USA) with a sample-to-detector distance of 40 mm. The sample solution of the same concentration as used at SACLA was circulated through a sapphire nozzle with a 300 µm-thick aperture. The sample flow velocity was set to be over 3 m s⁻ to supply fresh sample for every X-ray and laser shot. The sample in the reservoir was replaced with fresh sample whenever the reservoir sample failed to produce the transient signal measured at 100 ps. Even if the transient signal at 100 ps did not change, the sample in the reservoir was replaced with fresh one regularly (every 2-3 h of measurement) to ensure the delivery of fresh sample. The time resolution of the X-ray solution scattering experiment was 100 ps, which was limited by the duration of the X-ray pulses. The laser-off images were acquired with the X-ray pulse arriving 3 ns earlier than the laser pulse (that is, -3 ns time delay), to eliminate the contribution of the (unexcited) ground-state reactants. These laser-off images were used as a reference for calculating the time-resolved difference X-ray scattering patterns. To achieve a signal-to-noise ratio high enough for data analysis, more than 50 images were acquired and averaged at each time delay. The scattering curves were measured at the following time delays: -3 ns, -150 ps, 100 ps, 150 ps, 300 ps, 1 ns, 3 ns, 10 ns, 30 ns, 100 ns, 300 ns and 1 µs. The scattering signals arising from solvent (water) heating were also measured using 40 mM FeCl₃ solution with the same experimental conditions. Details of the data collection parameters are summarized in Supplementary Table 1.

Removal of the solvent contribution. To study only the dynamics of the Au–Au bond formation, the scattering arising from heating of pure solvent induced by laser excitation needs to be subtracted from the experimental scattering data. For this purpose, a separate time-resolved X-ray scattering experiment was performed on FeCl₃ solution in water. The resultant difference scattering curves are shown in Extended Data Fig. 2a. As can be seen in Extended Data Fig. 2b, c, SVD of the data identifies only one singular component, implying that only a single difference scattering curve accounts for the contribution of solvent heating in the time range up to 100 ps. In addition, as can be seen in Extended Data Fig. 2d, the difference scattering curve of the $[Au(CN)_2^-]_3$ solution at 1 µs time delay is identical to the one for solvent heating, confirming that the difference scattering curves of $[Au(CN)_2^-]_3$ at late time delays are dominated by the solvent heating contribution. The amount of heat dissipated in the sample solution can be determined from the scaling between these two curves. The obtained solvent heating contribution was subtracted from the experimental difference scattering curves at all time delays.

Fourier sine transformation of $q\Delta S(q)$ **.** The difference RDF, $r^2\Delta S(r, t)$, is a measure of the radial electron density change as a function of interatomic distance r in real space, and was obtained by Fourier sine transformation of the $q\Delta S(q, t)$ curves:

$$r^{2}\Delta S(r,t) = \frac{r}{2\pi^{2}} \int_{0}^{\infty} q\Delta S(q,t) \sin(qr) e^{-q^{2}\alpha} dq$$

Here the exponential with constant $\alpha = 0.03 \text{ Å}^2$ is a damping term that accounts for the finite *q* range in the experiment. The resultant $r^2 \Delta S(r,t)$ curves are shown in Extended Data Fig. 3.

Singular value decomposition. To determine the kinetic model of the photoinduced reaction of $[Au(CN)_2^-]_3$ and obtain the species-associated RDFs for each transient state, we applied the SVD analysis and kinetic analysis to our experimental RDFs. From the time-resolved X-ray scattering data, we can build an $n_r \times n_t$ data matrix, A, where n_r is the number of r points in the RDFs and n_t is the number of time-delay points. The matrix A can be decomposed into three matrices while satisfying the relationship $A = USV^T$, where U is an $n_r \times n_t$ matrix whose columns are called left singular vectors (that is, time-independent r-spectra) of A, V is an $n_t \times n_t$ matrix whose columns are called right singular vectors (that is, amplitude changes in the left singular vectors in U as time evolves) of A, and S is a diagonal $n_t \times n_t$ matrix whose diagonal elements are called singular values of A and can possess only non-negative values. The matrices U and V obey the relationships $U^T U = I_{n_t}$. and $V^T V = I_{n_t}$, respectively, where I_{n_t} is the $n_t \times n_t$ identity matrix. The diagonal elements of *S* (that is, singular values) represent the weights of left singular vectors in *U*. Because the singular values are ordered so that $s_1 \ge s_2 \ge ... \ge s_n \ge 0$ (both left and right), singular vectors on the left side of the matrix *U* are supposed to have larger contributions to the experimental TRXSS signal than are the ones on the right side of *U*. The left singular vectors, when linearly combined together, give information on the RDFs associated with distinct transient species. Thus, the SVD analysis provides a model-independent estimation of the number of structurally distinguishable transient species and the dynamics of each species.

By performing the SVD analysis on our experimental difference RDFs, $r^2\Delta S(r, t)$, we identified four singular components with significant singular values, indicating the existence of four structurally distinguishable transient states. The right singular vectors of these four significant singular components were fitted by a convolution of a Gaussian function representing the instrument response function (IRF) and a sum of three exponential functions representing transitions among the transient intermediate states. As a result, we obtained exponentials with time constants of 1.6 ± 0.1 ps, 3 ± 0.5 ns and 100 ± 20 ns and an IRF with a 480 ± 10 fs full-width at half-maximum. Thus, we identified four transient states and three kinetic components connecting the four species.

Kinetic analysis. To obtain species-associated RDFs of the four transient states identified in the SVD analysis, we performed kinetic analysis on the U and V matrices using an appropriate kinetic model. First, following the result of the SVD analysis, we defined new matrices, U', V' and S', that contain only the first four elements of U, V and S. In other words, U' is an $n_r \times 4$ matrix containing only the first four left singular vectors of U, S' is a 4×4 diagonal matrix containing only the first four singular values of *S*, and *V*' is an $n_t \times 4$ matrix containing the first four right singular vectors of V. Among various kinetic models, the only one that can account for the four transient states and the three kinetic components is the sequential model. Therefore, by solving rate equations based on the sequential model, the concentrations of the four transient states can be calculated using the three kinetic components determined from the SVD analysis. We defined a matrix C that represents the time-dependent concentrations of the four transient states and related it to V'using a parameter matrix P that satisfies the relation V' = CP. In other words, the linear combination (via P) of the concentrations of the four transient states (C) gives the four right singular vectors constituting V'. In our analysis, C is an $n_t \times 4$ matrix containing the time-dependent concentrations of the four transient states (S₀, S₁, T_1 and the tetramer), and P is a 4 \times 4 matrix containing coefficients that relate the time-dependent concentrations of the transient states in C to the right singular vectors in V'. Once we determine C by solving the rate equations and convolving with the IRF, the theoretical RDFs at various time delays, A', can be generated as follows:

$$A' = U'S'V'^{\mathrm{T}} = U'S'(CP)^{\mathrm{T}}$$

= U'S'(P^{\mathrm{T}}C^{\mathrm{T}}) = (U'S'P^{\mathrm{T}})C^{\mathrm{T}}(1)

The matrix *P* can be optimized by minimizing the discrepancy, χ^2 , between the theoretical and experimental difference scattering curves using the MINUIT package:

$$\chi^2 = \sum_{i=1}^{n_r} \sum_{j=1}^{n_i} \left(\frac{r^2 \Delta S_{\exp}(r_i, t_j) - r^2 \Delta S_{\text{theory}}(r_i, t_j)}{\sigma_{ij}} \right)^2$$

Here $r^2 \Delta S_{exp}(r_i, t_j)$ and $r^2 \Delta S_{theory}(r_i, t_j)$ are the experimental and theoretical RDFs at given r and t values, respectively, and σ_{ij} is the experimental standard deviation at given r and t values. From equation (1), we can define a matrix B as $B = U'S'P^{T}$, that is, a linear combination (via P) of the four left singular vectors constituting U' that are weighted by their singular values in S'. As a result, B, an $n_r \times 4$ matrix, contains the RDFs directly associated with the transient states. Thus, by optimizing P, we obtain the time-independent, species-associated RDFs of the intermediate species (optimized B).

Calculation of theoretical RDFs. For individual transient states, theoretical RDFs were expressed as a sum of multiple RDFs, S_{R_i} , each of which corresponds to an Au–Au pair:

$$r^{2}S_{\text{theory}}(r) = r^{2}\sum_{i=1}^{n}S_{R_{i}}(r)$$
 (2)

Here R_i is the Au–Au distance for the *i*th pair of Au atoms. For the trimer states (S₀, S₁ and T₁) and the tetramer, *n* was set to be 3 and 6, respectively. Each $S_{R_i}(r)$ curve was calculated by Fourier sine transformation of the theoretical scattering curve, $S_{R_i}(q)$, as follows:

$$r^{2}S_{R_{i}}(r) = \frac{r}{2\pi^{2}} \int_{0}^{\infty} qS_{R_{i}}(q) \sin(qr) e^{-q^{2}\alpha} dq$$
(3)

The damping constant (α) used to obtain the experimental RDFs was also used to obtain the theoretical RDFs. The theoretical scattering curves $S_{R_i}(q)$ from Au–Au pairs were obtained by the simple Debye formula

$$S_{R_i}(q) = F_{Au}^2(q) \frac{\sin(qR_i)}{qR_i}$$
(4)

where F_{Au} is the atomic form factor of the Au atom. By substituting equations (3) and (4) into equation (2), we obtained

$$r^{2}S_{\text{theory}}(r) = \sum_{i=1}^{n} \frac{r}{2\pi^{2}} \int_{0}^{\infty} qF_{Au}^{2}(q) \frac{\sin(qR_{i})}{qR_{i}} \sin(qr) e^{-q^{2}\alpha} \,\mathrm{d}q$$
(5)

The theoretical RDFs for the transient states were calculated by equation (5). We note that the only variables in equation (5) are the Au-Au distances.

Structural fitting analysis. To reconstruct the structures of the four states (S₀, S₁, T₁ and the tetramer) and extract the Au–Au distances for each state, we performed a structural fitting analysis of the species-associated RDFs of the four states. As fitting parameters of the analysis, we considered three Au–Au distances for the S₀, S₁ and T₁ states, six Au–Au distances for the tetramer, and a scaling factor between the number of excited molecules and the signal intensity. The maximum-likelihood estimation with the χ^2 estimator^{15,36,37} was used with four (for S₀, S₁ and T₁) or seven (for the tetramer) variable parameters. The χ^2 estimator is given by

$$\chi^{2}(R_{1},R_{2},R_{3},A) = \frac{1}{N-p-1} \sum_{i} \frac{(S_{\text{theory}}(r_{i}) - S_{\exp}(r_{i}))^{2}}{\sigma_{i}^{2}}$$

where N = 500 is the total number of r points, p = 4 or 7 is the number of fitting parameters and σ_i is the standard deviation. The likelihood (*L*) is related to χ^2 by the following equation:

 $L(R_1, R_2, R_3, A) \propto \exp(-\chi^2/2)$

The errors of multiple fitting parameters can be determined from this relationship by calculating the boundary values at 68.3% of the likelihood distribution. The calculation was performed by the MINUIT software package, and the error values were provided by MINOS algorithm in MINUIT. Because we used the standard deviation of the measurement when calculating χ^2 , the quality of the fit becomes better as χ^2 approaches 1. Figure 2d shows the fitting results for the four transient states as well as their reconstructed structures based on the optimized Au–Au distances.

Determination of RDF of the S₀ **state.** As shown in Extended Data Fig. 4, timeindependent, species-associated difference RDFs of the transient states were obtained by the SVD and kinetic analyses of the experimental, time-dependent, difference RDFs (Extended Data Fig. 3). These difference RDFs correspond to $r^2(S_{S_0}(r) - S_{S_0}(r))$, $r^2(S_{S_1}(r) - S_{S_0}(r))$, $r^2(S_{T_1}(r) - S_{S_0}(r))$ and $r^2(S_{tetramer}(r) - S_{S_0}(r))$ as indicated in Extended Data Fig. 4. From the structural fitting analysis, we were able to determine not only the RDFs of the transient states (S₁, T₁ and the tetramer) but also the RDF of the S₀ state. We used a common S₀ structure when fitting the four speciesassociated difference RDFs. By optimizing the fit between the experimental and the theoretical difference RDFs for each transient species, we were able to obtain the theoretical BDF of the S₀ state in addition to the RDFs of the other states. To emphasize only the contributions of transient solute species associated with the bond formation, we added the RDF of the S₀ state to the experimental difference RDFs at all time delays and obtained the RDFs $r^2S(r, t)$ shown in Extended Data Fig. 5.

- Inubushi, Y. *et al.* Determination of the pulse duration of an X-ray free electron laser using highly resolved single-shot spectra. *Phys. Rev. Lett.* **109**, 144801 (2012).
- Ishikawa, T. et al. A compact X-ray free-electron laser emitting in the sub-angstrom region. Nature Photon. 6, 540–544 (2012).
- Rawashdeh-Omary, M. A., Omary, M. A. & Patterson, H. H. Oligomerization of Au(CN)₂⁻ and Ag(CN)₂⁻ ions in solution via ground-state aurophilic and argentophilic bonding. J. Am. Chem. Soc. **122**, 10371–10380 (2000).
- Kim, T. K., Lee, J. H., Wulff, M., Kong, Q. Y. & Ihee, H. Spatiotemporal kinetics in solution studied by time-resolved X-ray liquidography (solution scattering). *ChemPhysChem* 10, 1958–1980 (2009).
- Ichiyanagi, K. et al. 100 ps time-resolved solution scattering utilizing a widebandwidth X-ray beam from multilayer optics. J. Synchrotron Radiat. 16, 391–394 (2009).
- Haldrup, K. et al. Structural tracking of a bimolecular reaction in solution by timeresolved X-ray scattering. Angew. Chem. Int. Ed. 48, 4180–4184 (2009).
- Jun, S. et al. Photochemistry of HgBr₂ in methanol investigated using timeresolved X-ray liquidography. *Phys. Chem. Chem. Phys.* **12**, 11536–11547 (2010).

RESEARCH LETTER



Extended Data Figure 1 | **Comparison of the TRXSS signals at SACLA and KEK and the TRXSS data in the entire time range. a**, Comparison of the difference scattering curves at 100 ps time delay measured at SACLA (black) and KEK (red). The error bar at each data point indicates the standard error determined from 50 independent measurements. The two curves are nearly

identical to each other within the experimental error, indicating that the difference scattering curves are highly reproducible and independent of the facility. **b**, Experimental difference scattering curves, $q\Delta S(q, t)$, in the entire time range from -800 fs to 1 µs.



Extended Data Figure 2 Solvent heating contribution to the TRXSS signal. a, Experimental difference scattering curves, $q\Delta S(q)$, of FeCl₃ solution measured at several time delays (400 fs, 1.9 ps, 3.9 ps, 5.9 ps, 7.9 ps, 30 ps and 100 ps). **b**, SVD of the experimental difference scattering curves of FeCl₃ measured from –10 ps to 100 ps. The first two right singular vectors multiplied by singular values are shown. c, The first right singular vector (black circles) fitted by an error function (red curve). This result implies that only a single difference scattering curve accounts for solvent heating in the time range up to

100 ps. **d**, Comparison of the difference scattering curve of the $[Au(CN)_2]_3$ solution at 1 µs time delay (black) and the difference scattering curve for solvent heating (red). The error bar at each data point indicates the standard error determined from 50 independent measurements. At this time delay, the two curves are almost identical to each other within the experimental error, confirming that the difference scattering at late time delays are dominated by the solvent heating.



Extended Data Figure 3 | **Difference RDFs in real space.** Difference RDFs, $r^2\Delta S(r)$, obtained by Fourier sine transformation of $q\Delta S(q)$.



Extended Data Figure 4 | **Species-associated difference RDFs of the transient states.** The species-associated difference RDFs of the S₀, S₁, T₁ and tetramer states correspond to $r^2(S_{S_0}(r) - S_{S_0}(r))$, $r^2(S_{S_1}(r) - S_{S_0}(r))$, $r^2(S_{T_1}(r) - S_{S_0}(r))$ and $r^2(S_{tetramer}(r) - S_{S_0}(r))$, respectively. We used a common S₀ structure when fitting all four species-associated difference RDFs. By optimizing the fit between the theoretical and the experimental difference RDFs for each transient species via the structural fitting analysis, we were able to obtain the theoretical RDF of the S₀ state.



Extended Data Figure 5 | **Radial distribution functions**, $r^2S(r, t)$. The RDF of the S₀ state was added to the RDFs at all time delays to emphasize only the contributions of the transient solute species associated with the bond formation.



Extended Data Figure 6 Comparison of the scattering from Au atoms and other contributions. a, Because the scattering intensities from C and N atoms are negligibly small, the total scattering pattern is almost the same as the scattering from Au atoms only. **b**, The contribution of the cage term is small and the total scattering pattern is therefore almost the same as the solute-only term.



Extended Data Figure 7 | **Contributions of trimer and dimer to X-ray scattering signal. a**, Concentrations of the three species [Au], [Au₂] and [Au₃], calculated as a function of *c*, which is the initial concentration of monomers of the gold complex. We assumed that K_2 is 10 M^{-2} in this case. **b**, Absorption spectra of aqueous solutions of K[Au(CN)₂] at various concentrations measured with a 0.5 mm path length cell. Four points (A₁, A₂, A₃ and A₄) that are used as inputs are indicated. **c**, Theoretical difference scattering curves for the trimer (black) and the dimer (red). Relative intensities of the two curves were estimated realistically based on the excitation probabilities and the equilibrium of the two species.



Extended Data Figure 8 Comparison of the TRXSS data measured with excitations at two different wavelengths, 310 and 267 nm. a, Right singular vectors obtained from the SVD analysis for the 310 nm (left) and 267 nm (right) excitations. For both cases, the right singular vectors were fitted by only one kinetic component and their extracted time constants are almost identical to each other. b, Difference scattering curves at 100 ps time delay measured

with excitations at 310 nm (black) and 267 nm (red). The error bar at each data point indicates the standard error determined from 50 independent measurements. The two curves are identical to each other within the experimental error. This similarity between the kinetics and the shapes of the difference scattering curves indicates negligible contribution from dimer excitation for both 310 and 267 nm excitations.





Reaction progress

Extended Data Figure 9 | Mechanism of photoinduced bond formation in $[Au(CN)_2^-]_3$. Results from our TRXSS data (red) and the previous transient absorption experiment (blue) are shown together. Our findings are in good agreement with the reaction mechanism proposed in the transient absorption study, except for the structural assignments of the early kinetics.

Considering that TRXSS is sensitive only to the processes accompanying structural change, the intersystem crossing processes on $\sim\!500$ fs and 13 ns timescales, which were not observed in the TRXSS measurement, are likely to involve no structural change.



Extended Data Figure 10 | Species-associated RDFs of the four structures obtained from the SVD and principal-component analyses (black) and their fits (red) obtained by using the Debye–Waller factor and the constraint of the symmetric structure for the S_0 state. For each state, the structural parameters obtained from the fits and their standard errors determined from 50 independent measurements are shown together. It can be seen that the structural parameters of S_1 , T_1 and the tetramer obtained from the fits using the Debye–Waller factor and the symmetric constraint for S_0 are similar to the values given in Fig. 2d.

Beamline	SACLA	SACLA	KEK
Date	2014. 4	2013. 9	2013. 5
X-ray energy (keV)	15.0	15.0	15.6
X-ray energy band width (%)	0.6	0.6	5
X-ray Size (mm ²)	0.2 × 0.2	0.09 × 0.08	0.2×0.2
X-ray fluence (mJ/mm ²)	1.30	1.87	0.017
X-ray pulse duration	< 100 fs	< 100 fs	100 ps
Spatial fluctuation (%)	10	10	~ 0
Repetition rate (Hz)	30	20	946
Laser wavelength (nm)	267	310	267
Laser pulse energy (µJ)	150	18	259
Laser size (vertical × horizontal, mm ²)	0.30 × 0.30	0.095 × 0.12	0.30 × 0.30
Laser fluence (mJ/mm ²)	2.12	2.05	3.67
Laser pulse duration	100 fs	100 fs	150 fs
Liquid jet thickness (mm)	0.1	0.1	0.3
Angle between X-ray and Laser (°)	10	10	10
Detector	Rayonix_LX255- HS	Rayonix_LX255- HS	MarCCD_165

Extended Data Table 1 | Details of the data-collection parameters