Time-Resolved Dimerization of a PAS-LOV Protein Measured with Photocoupled Small Angle X-ray Scattering

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · OCTOBER 2008

Impact Factor: 12.11 · DOI: 10.1021/ja804236f · Source: PubMed

5 AUTHORS, INCLUDING:

Jessica S Lamb
U.S. Department of Health and Human Services
23 PUBLICATIONS **489** CITATIONS
SEE PROFILE

Brian D Zoltowski
Southern Methodist University
35 PUBLICATIONS **661** CITATIONS
SEE PROFILE

Suzette A Pabit
Cornell University
41 PUBLICATIONS **954** CITATIONS
SEE PROFILE

Published in final edited form as:
J Am Chem Soc. 2008 September 17; 130(37): 12226–12227. doi:10.1021/ja804236f.

Time-resolved dimerization of a PAS-LOV protein measured with photocoupled small angle x-ray scattering

Jessica S. Lamb, **Brian D. Zoltowski**, **Suzette A. Pabit**, **Brian R. Crane**, and **Lois Pollack***
School of Applied and Engineering Physics and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853

Abstract

Time-resolved small angle x-ray scattering (SAXS) has been used to probe photoexcitation of the blue-light signal transduction protein Vivid (VVD). Laser excitation of sample in a continuous flow cell enables time-resolved measurement of the initial response of VVD to illumination. Good signal-to-noise is achieved without relying on multiple exposures of the same sample or limiting exposure times to prevent radiation damage. The SAXS data demonstrate that VVD dimerizes within tens of milliseconds of light-state activation. Time-resolved SAXS in a flow cell format is a general method for connecting chemical changes in photoreceptors to conformationally driven output signals.

Here, we report the coupling of photoexcitation with time-resolved SAXS to monitor conformational changes accompanying light activation of the blue-light signal transduction protein Vivid (VVD). Solution small angle X-ray scattering (SAXS) reports the size and shape of soluble biomolecules. Previously, SAXS was used in conjunction with rapid mixing techniques to time resolve macromolecular folding[1,2]. SAXS studies have also been used to determine large scale differences between the dark and light excited states of proteins[3,4]. Flash-flow devices have been used for time-resolved IR spectroscopy[5]; integration with SAXS expands the technique, enabling exploration of global kinetics. While time dependent processes in different blue-light sensors have been revealed by a variety of techniques[6,7], this method elucidates previously unknown association dynamics of the long-lived VVD light-adapted state.

VVD, a so-called LOV (for Light Oxygen Voltage sensing) protein of the PAS family, regulates blue-light responses in the filamentous fungus *Neurospora crassa* 8. Photon absorption by the VVD flavin cofactor drives conformational changes within the LOV domain. In the absence of light, the protein is monomeric. Recent measurements suggest the establishment of a rapidly exchanging monomer:dimer equilibrium in the light-activated state[9], thus VVD (like other LOV domains[10,11]) changes association state in response to light-stimulated structural modifications. PAS:PAS dimerization is believed to be a key regulatory event in signal transduction[12], and is likely important for a close VVD homolog, WC-1, to activate transcription[13]. Because of the challenge of characterizing structural intermediates in these processes, little is known about how cofactor chemical state relates to association mode, and ultimately, the engagement of targets.

To time-resolve changes in the structure of VVD resulting from photoexcitation, we employed a microfluidic cell coupled to both laser and x-ray sources (Figure 1). The cell consists of a thin wall polyester tube (Advanced Polymers, VT)[14] which is optically transparent and scatters x-rays minimally. A 473 nm laser beam (Holograms & Lasers International, Texas) is directed

lp26@cornell.edu.

at 90° to a focused x-ray beam. The location where the x-ray and light beams intersect defines the time-equals-zero position for the experiment. Offsetting the two beams creates a controllable delay between laser excitation and SAXS measurement. This average delay time is calculated by dividing the distance between the two beams, *x*, by the flow speed of the protein solution, *u* (Figure 1). Use of a flow cell eliminates radiation damage to the protein and enables long x-ray exposures, which increase signal-to-noise without loss of time resolution. Temporal precision is restricted by the laminar flow boundary condition at the channel wall where $u=0$, but can be improved by employing a flow of sheath buffer which confines the protein-containing solution to a thin jet that travels along the tube axis with relatively uniform speed. The transit time of each molecule through either the laser or x-ray incident spot was 160 ms on average. The laser spot was continuously illuminated for all light-state measurements and the x-ray exposure time ranged from 20 to 30 sec.

The intensity of the scattered x-rays is typically plotted as a function of the momentum transfer $q = 4\pi \sin(\theta)/\lambda$ where θ is half the scattering angle and λ is the x-ray wavelength. Following standard analysis procedures[17] the zero angle intensity, I(0), and the radius of gyration, (R_g), were extracted from the low *q* data (see supplemental information). A Kratky plot[18] places emphasis on data acquired at large *q*, which provides detail about macromolecular conformation.

SAXS measurements of the dark state of VVD are in good agreement with the computed signal from the monomeric crystal structure 2PD7 (supplementary information)[4]. Upon light illumination, the low angle scattering intensity from VVD is increased relative to the dark state (Fig. 2 and supplementary information). The increase occurs rapidly, and appears finished by the earliest time detected, 20 ms after photoexcitation. Such an increase in I(0) is consistent with dimerization. Inline multi-light scattering (MALS), dynamic light scattering (DLS), size exclusion chromatography and equilibrium ultracentrifugation confirm the presence of a rapidly dissociating dimer in the light adapted state[9]. Complete dimerization in 20 msec is consistent with a diffusion controlled association rate constant in the range of 10^5–10^6 M^{-1} s^{-1} [9], and demonstrates that the conformational change which precedes dimerization is unlikely to be rate limiting. This association rate exceeds that reported for other LOV domain proteins[11]. Importantly, we also evaluated the time-dependent SAXS profile of a VVD point mutant (Cys71Ser) that cannot undergo light-induced dimerization, but otherwise has normal photochemical properties[4]. Cys71Ser VVD undergoes no change in scattering upon illumination; thus, laser heating or radiation damage cannot be the cause of the scattering changes we observe with wild-type VVD.

Scattering data acquired at longer times after photoexcitation show variation in the high *q* region of the profile, suggesting that the conformational changes continue for several seconds after dimerization is complete (Figure 3). As VVD remains in the light-adapted state for several hours[19] this phenomenon reflects additional changes *post* dimerization that may be essential for engaging targets and propagating signals.

While these time-resolved SAXS measurements show a consistent pattern of oligomerization after light excitation, the magnitude of the change varies depending on the specific sample and experiment. The affinity of the VVD dimer is known to be highly sensitive to modifications at the N-terminus as well as protein oxidation at currently unspecified sites[9]. We suspect differences in dimer yield result from such subtle changes in protein chemistry; we are exploring the phenomenon in more detail. Measurements on millisecond (or sub-millisecond) time scales will be required to directly detect light-induced conformational differences in the monomeric state which must precede and gate access to the dimeric state

Time-resolved measurements of global structural states are critical for unraveling the protein dynamics that underlie LOV protein light sensing; SAXS is an ideal tool for studying both the conformational changes and protein association that accompany such events. Application of a continuous flow cell to couple SAXS and photoexcitation, as demonstrated here, can elucidate ~millisecond conformational changes without signal averaging by photocycle repetition, thus avoiding the sample damage associated with repeat exposures and facilitating measurement of samples with slow photocycles. Notably, this method will resolve diffusion-limited association rates and it is generally applicable to many photoreceptors, even those with transient light-adapted states much less stable than that of VVD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

We thank Arthur Woll, Kurt Andresen, Xiangyun Qiu, Li Li, and Sterling Cornaby for help with data collection. This work was supported by the Cornell Nanobiotechnology Center (NSF ECS-9876771) NSF grant MCB-0347220 NIH grant GM079679. This research was conducted at the Cornell High Energy Synchrotron Source (CHESS) which is supported by the NSF and the NIH..

REFERENCES

1. Moody MF, Vachette P, Foote AM, Tardieu A, Koch MHJ, Bordas J. Proc. Natl. Acad. Sci. U. S. A 1980;77(7):4040–4043. [PubMed: 6933451]
2. Pollack L, Tate MW, Darnton NC, Knight JB, Gruner SM, Eaton WA, Austin RA. Proc. Natl. Acad. Sci. U. S. A 1999;96(18):10115–10117. [PubMed: 10468571]
3. Nakasako M, Iwata T, Matsuoka D, Tokutomi S. Biochemistry 2004;43(47):14881–14890. [PubMed: 15554695]
4. Zoltowski BD, Schwerdtfeger C, Widom J, Loros JJ, Bilwes AM, Dunlap JC, Crane BR. Science 2007;316(5827):1054–1057. [PubMed: 17510367]
5. Toepke MW, Brewer SH, Vu DM, Rector KD, Morgan JE, Gennis RB, Kenis PJA, Dyer RB. Anal. Chem 2007;79(1):122–128. [PubMed: 17194129]
6. Harper SM, Neil LC, Day IJ, Hore PJ, Gardner KH. J. Am.Chem. Soc 2004;126(11):3390–3391. [PubMed: 15025443]
7. Kennis JTM, van Stokkum NHM, Crosson S, Gauden M, Moffat K, van Grondelle R. J. Am. Chem. Soc 2004;126(14):4512–4513. [PubMed: 15070357]
8. Loros JJ, Dunlap JC. Annu. Rev. Physiol 2001;63:757–794. [PubMed: 11181975]
9. Zoltowski BD, Crane BR. Biochemistry 2008;47(27):7012–7019. [PubMed: 18553928]
10. Harper SM, Neil LC, Gardner KH. Science 2003;301(5639):1541–1544. [PubMed: 12970567]
11. Nakasone Y, Eitoku T, Matsuoka D, Tokutomi S, Terazima M. Biophys. J 2006;91:645–653. [PubMed: 16679373]
12. Card PB, Erbel PJA, Gardner KH. J. Mol. Biol 2005;353(3):664–677. [PubMed: 16181639]
13. Froehlich AC, Liu Y, Loros JJ, Dunlap JC. Science 2002;297(5582):815–819. [PubMed: 12098706]
14. Kalinin Y, Kmetko J, Bartnik A, Stewart A, Gillilan R, Lobkovsky E, Thorne R. J. Appl. Crystallogr 2005;38:333–339.
15. Engstrom P, Larsson S, Rindby A, Buttkewitz A, Garbe S, Gaul G, Knochel A, Lechtenberg F. Nucl. Instrum. Methods Phys. Res.,Sect. A 1991;302(3):547–552.
16. Lamb JS, Cornaby S, Andresen K, Kwok L, Park HY, Qiu XY, Smilgies DM, Bilderback DH, Pollack L. J. Appl.Crystallogr 2007;40:193–195.
17. Guinier, A.; Fournet, G. Small-Angle Scattering of X-Rays. New York: John Wiley and Sons., Inc.; 1955. p. 216
18. Glatter, O.; Kratky, O. Small Angle X-ray Scattering. London: Academic Press; 1982.

19. Schwerdtfeger C, Linden H. EMBO J 2003;22(18):4846–4855. [PubMed: 12970196]

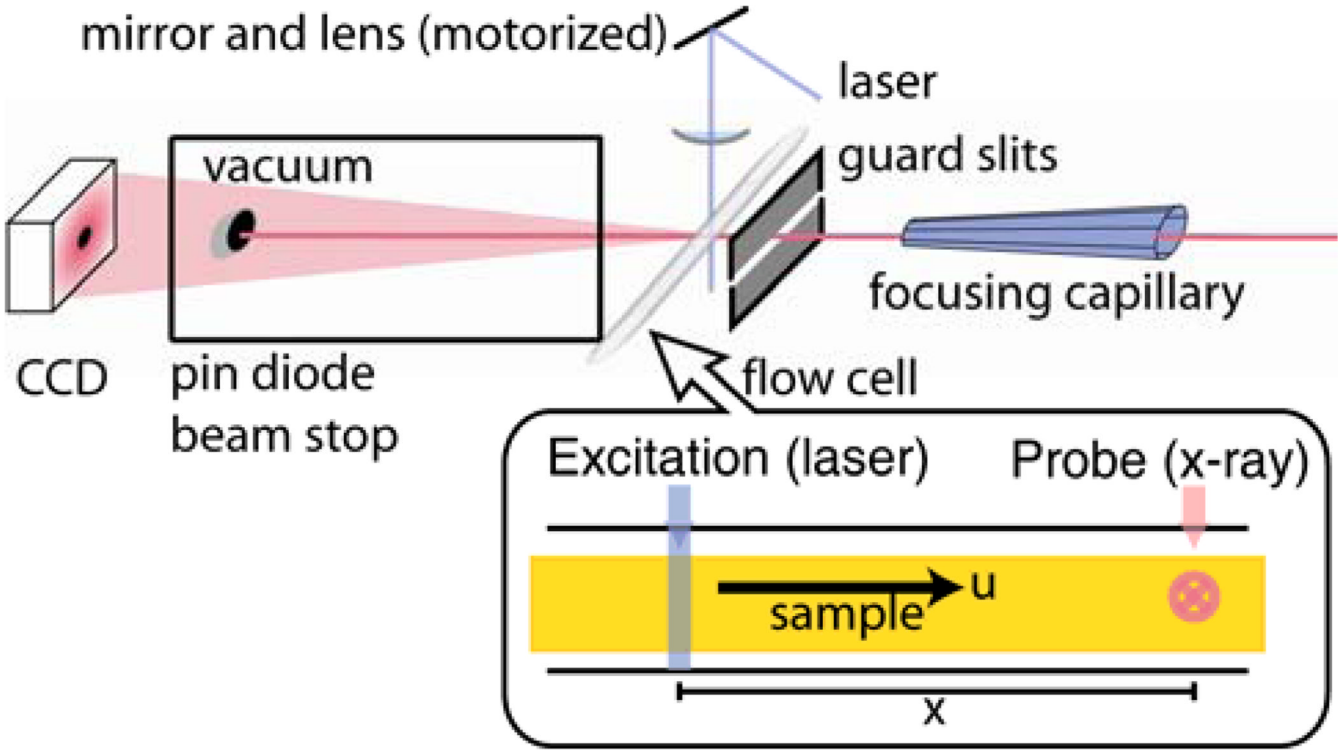

Figure 1.
A schematic of the continuous flow setup that enables time-resolved SAXS measurements following photoexcitation of protein. An x-ray beam, incident from the right, passes through a focusing capillary[15,16] to obtain sufficient x-ray flux in a small spot. Guard slits are used to decrease the scattering background. The excitation laser beam is directed perpendicular to the axis of the flow cell and the x-ray beam. A magnified illustration of the flow cell demonstrates how time-resolution is achieved.

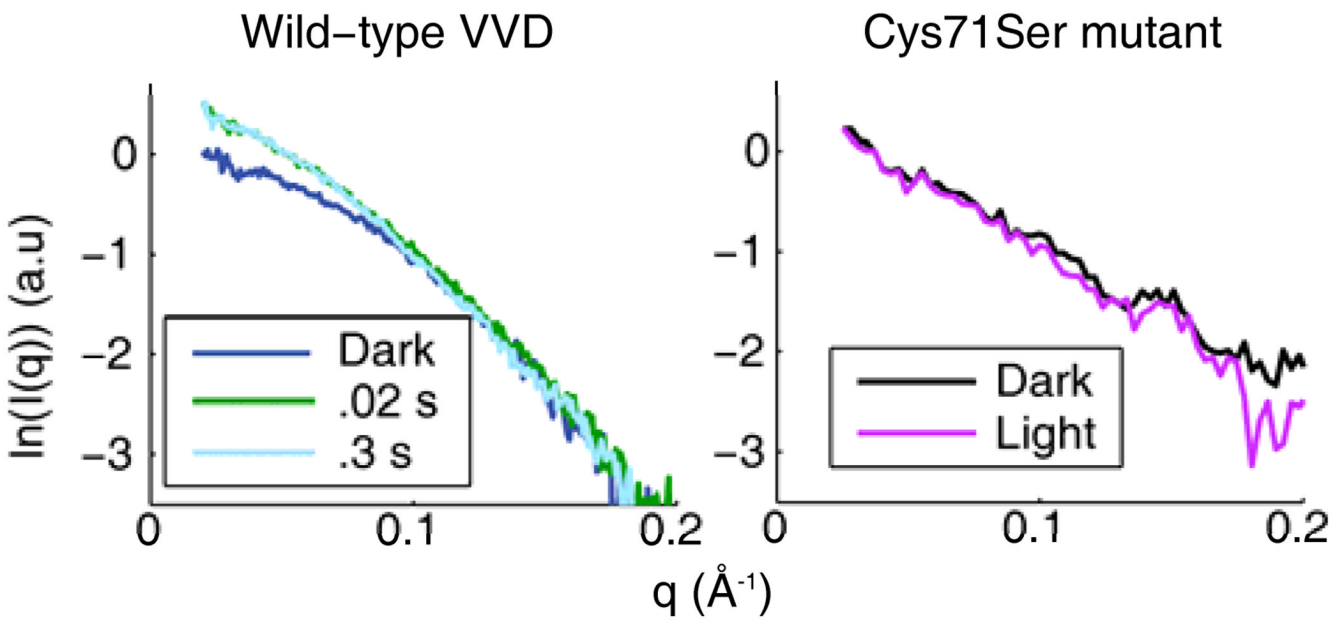

Figure 2.
Left: Scattering profiles for wild-type VVD before (blue) and 0.02 s after excitation (green) display an increase in zero angle intensity, consistent with light induced dimerization. No further change is measured 0.3 s after excitation (cyan). Right: Scattering profiles of the inactive Cys71Ser mutant are not changed by light excitation.

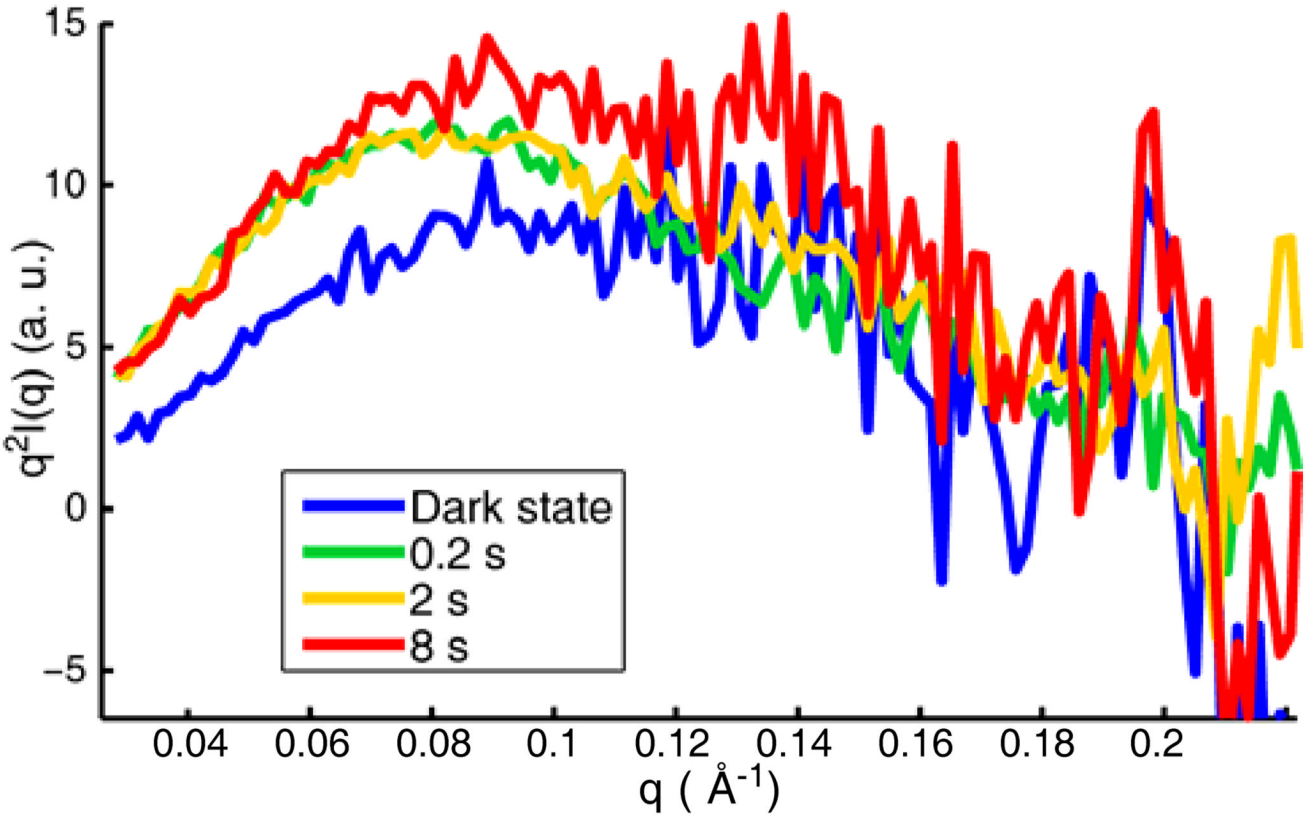

Figure 3.
Kratky plots of data collected hundreds of milliseconds to seconds after photoexcitation. Variations in scattering profiles become evident at q>0.08 Å^{-1} after 8 s indicating conformational changes of the dimer.