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Highly Sensitive SERS Quantification of the Oncogenic Protein c-Jun in Cellular Extracts

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Supporting Information

ABSTRACT: A surface-enhanced Raman scattering (SERS)-based sensor was developed for the detection of the oncoprotein c-Jun at nanomolar levels. c-Jun is a member of the bZIP (basic zipper) family of dimeric transcriptional activators, and its overexpression has been associated with carcinogenic mechanisms in several human cancers. For our sensing purpose, we exploited the ability of c-Jun to heterodimerize with its native protein partner, c-Fos, and therefore designed a c-Fos peptide receptor chemically modified to incorporate a thiophenol (TP) group at the N-terminal site. The TP functionality anchors the c-Fos protein onto the metal substrate and works as an effective SERS probe to sense the structural rearrangements associated with the c-Fos/c-Jun heterodimerization.

Transcription factors are proteins that bind to specific regulatory regions of the genes and thereby control the initiation of their transcription by the RNA polymerase.[1] It is well known that many anomalous cell processes, including abnormal cellular proliferations related to a neoplastic transformation, are intimately related to the aberrant expression of some of these transcription factors.[2] This is the case with the oncoprotein c-Jun, a component of the AP-1 transcription complex that has been associated with carcinogenic mechanisms in several human cancers.[3−9] Indeed, it has been shown that a large number of malignant transformations present elevated levels of c-Jun expression,[4,10,11] although not much quantitative information has been reported to date (due to the lack of appropriate analytical methods). c-Jun content varies with the cellular line under study from nanomolar to micromolar and, generally, overexpressions over 1 order of magnitude during carcinogenic processes are commonly reported.[10] Therefore, a prompt and reliable detection of this oncoprotein in its complex biological media may have great diagnostic and therapeutic impact, and aid in further studies on its biological role. Conventional methods for identification of transcription factors, such as electrophoretic mobility shift assays[12] and DNase footprinting assays,[13] are quite laborious and time-consuming. Thus, many efforts are being devoted to develop analytical tools capable of overcoming these limitations, including, among others, colorimetric sandwich assays,[14] protein binding microarrays,[15] and chromatin immunoprecipitation chips.[16] All of these methods rely on the use of relatively elaborated DNA constructs, and most of them suffer from low sensitivity or selectivity. In this regard, we have recently developed a sensing strategy to detect the DNA-binding domain of c-Jun on the basis of the increase in luminescence of a designed c-Fos receptor when forming the coiled coil heterodimer with c-Jun.[17] The method avoids the use of DNA receptors, but its sensitivity is relatively low.

In recent years, surface-enhanced Raman scattering (SERS) spectroscopy has emerged as one of the most powerful analytical techniques for the fast and sensitive interrogation of specific targets.[18−20] SERS retains the rich chemical and structural information provided by Raman spectroscopy but overcomes its inherent limitation to the investigation of low amounts of material by exploiting the enormous electromagnetic field enhancement resulting from the excitation of localized surface plasmon resonances at nanostructured metallic surfaces (mostly gold or silver).[21,22] Additionally, the existence of surface selection rules[23] imposes different enhancements for the Raman features depending on the symmetry of the corresponding vibrational modes. This provides a valuable tool to investigate possible preferential orientations of the molecule onto the metal surface. However, while the direct SERS measurement of the intrinsic Raman spectra of small molecules is relatively straightforward, several limitations in sensitivity, selectivity, and reproducibility often arise for biological targets with high molecular weight, such as proteins.[24] A potential solution to detecting such large biomolecules is based on the introduction into the sensing system of Raman labels with intense and characteristic SERS signals that undergo distinctive changes upon recognition of the biomolecular target. Usually, Raman labels have been installed at the metal substrate together with the biomolecule receptor, and the analyte recognition event is revealed by intensity changes of the reporter SERS signal.[25−28] Alternatively, the analyte recognition can induce characteristic spectral changes of the label SERS profile, which usually arise from re-orientation

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of the label with respect to the plasmonic surface (i.e., surface selection rules) and/or deformation of its molecular structure (i.e., rearrangement of the electronic cloud).[29] In this Communication, we describe a SERS sensor for highly sensitive detection of c-Jun on a bio-relevant aqueous media. The sensor features the DNA binding domain of a natural c-Jun partner (c-Fos) and a suitable SERS reporter attached at its N-terminus, and allows the specific detection of minute amounts of the target.

It is well known that in bZIP transcription factors like AP-1, c-Jun and c-Fos dimerize via a leucine zipper domain, a process which promotes the folding of the largely unstructured monomeric species into α-helix secondary structures in the homo-/heterodimers.[30] We envisioned that this drastic conformational change could be exploited for our sensing purposes by designing a c-Fos peptide receptor chemically modified by covalent attachment of a thiophenol (TP) group at the N-terminal amino acid residue. The TP functionality not only acts as a strong anchoring group for attaching the protein onto the metal substrate via a covalent bond but it also provides a very intense, highly reproducible, and fully vibrationally characterized SERS spectrum that might effectively sense the structural rearrangements associated to the c-Fos/c-Jun heterodimerization. In this regard, the selection of a single benzene ring as a direct molecular spring joining the c-Fos protein with the metal surface, is expected to maximize the possible mechanical deformations of the SERS probe structure upon c-Jun complexation (i.e., maximum sensitivity). As c-Jun receptor we chose a peptide featuring the dimerization (leucine zipper) and basic region of the c-Fos DNA binding domain (amino acids 138−193).[17,31,32]

Silver nanoparticles (AgNPs), prepared via chemical reduction with sodium citrate, were self-assembled on 3-(aminopropyl)triethoxysilane-modified glass slides (Figure 1A) and then functionalized with the TP-labeled c-Fos synthetic derivative (c-FosTP). In a subsequent step, octanethiol, a molecular probe characterized by its low Raman cross-section,[33] was assembled to fill in any gaps within the c-FosTP monolayer. The alkanethiol surface functionalization is intended, first, to minimize direct interactions of the large c-Fos protein moiety with the metal and thereby induce the adoption of a preferential upright position and, second, to prevent nonspecific binding of the target c-Jun and the components of the biological medium to the metal. The so-prepared sensing platform was then stored in HEPES buffer before being incubated with c-Jun (DNA binding domain, see Supporting Information) solutions at different concentrations.

AgNPs film functionalized with c-FosTP shows the intense SERS features characteristic of the TP molecule[34] (Figure 2A) such as the bands at 1585 cm^{-1}, ascribed to the aromatic ν(CC) modes, at 1075 cm^{-1}, assigned to an in-plane ring breathing mode coupled with ν(C−S), at 1022 and 998 cm^{-1}, both resulting from in-plane ring breathing vibrations, at 691 cm^{-1}, attributed to C−H out-of-plane deformation, and at 417 cm^{-1}, assigned to the ν(C−S) mode. Upon exposure of the c-FosTP-metal substrate to c-Jun solutions in HEPES buffer (20 mM HEPES pH 7.6, 60 mM NaCl) at different concentrations, a re-shaping of the SERS spectral profile takes place. The most prominent changes are observed for the bands at 1075 and 1585 cm^{-1}. This is not surprising since the vibrational modes associated with these bands are known to show the largest interfacial contribution to the change in polarizability of the phenyl ring (Ph) in the TP−metal surface complex.[35] Such

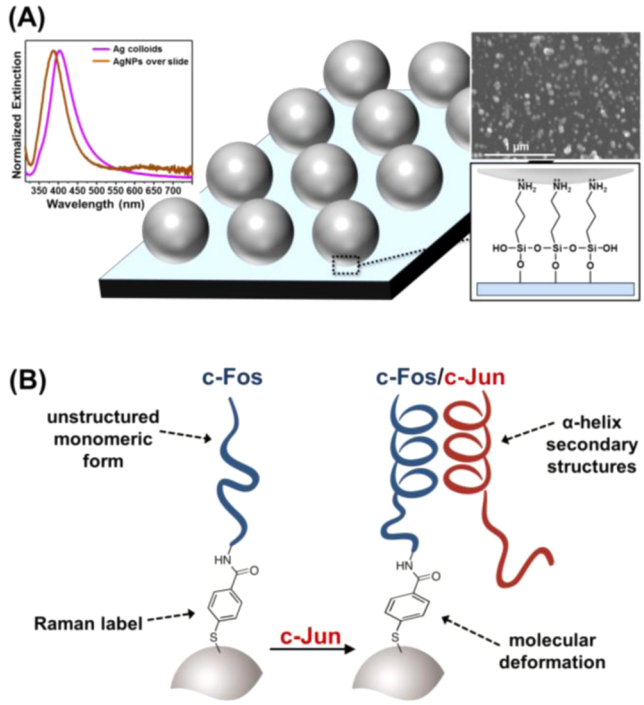

Figure 1. (A) Schematic representation of the SERS substrate (AgNPs over silanized glass slide) including the normalized extinction spectra of AgNPs in solution and deposited on the glass substrate. A representative SEM image of the film is also shown. (B) Outline of the c-Fos/c-Jun dimerization on the metal surface and the resulting deformation of the Raman label structure. c-Fos peptide sequence: MKRRIRRERNKMAAAKCRNRRRELTDTLQAETDQLEDEKSAL-QTEIANLLKEKEKLW.

intrinsic property is at the heart of the largest enhancement and marked spectral downshifts that these Raman features exhibit upon adsorption of TP onto the metal.[35,36] For the same reason, the contributions at 1075 and 1585 cm^{-1} are truly spectral markers for monitoring deformations of the electronic molecular structure of the Ph−S−metal surface complex caused by external factors such as binding events involving a substituent group of the phenyl ring[29] (as for the c-Fos/c-Jun dimerization).

In the case of the in-plane ring breathing mode coupled with ν(C−S), the corresponding Raman band exhibits a progressive downshift with increasing c-Jun concentrations (Figure 2B), whereas in the aromatic CC stretching region (Figure 2D) we observe a drastic increase in the relative intensity of the weak shoulder at ca. 1574 cm^{-1}, corresponding to the non-totally symmetric CC vibration,[37] with respect to the totally symmetric mode at 1585 cm^{-1}.[37] Such redistribution of the Raman scattering cross sections among the aromatic ν(CC) vibrations is theoretically attributed to the perturbation of the C_s symmetry of the benzene ring of the Ph−S−Ag surface complex.[37] Additionally, the normalized SERS spectra illustrated in Figure 2A show a rather uniform intensity increase of the in-plane vibrations at 417, 998, and 1022 cm^{-1} and of the C−H out-of-plane deformation at 691 cm^{-1} with respect to the two marker bands (Figure S2). The unspecific intensity increase of these in-plane and out-of-plane modes suggests that the overall spectral changes observed upon c-Jun addition are largely determined by the modification of the electronic polarizability of the Raman probe as a consequence of the c-Fos/c-Jun dimerization, rather than by a mere re-orientation of

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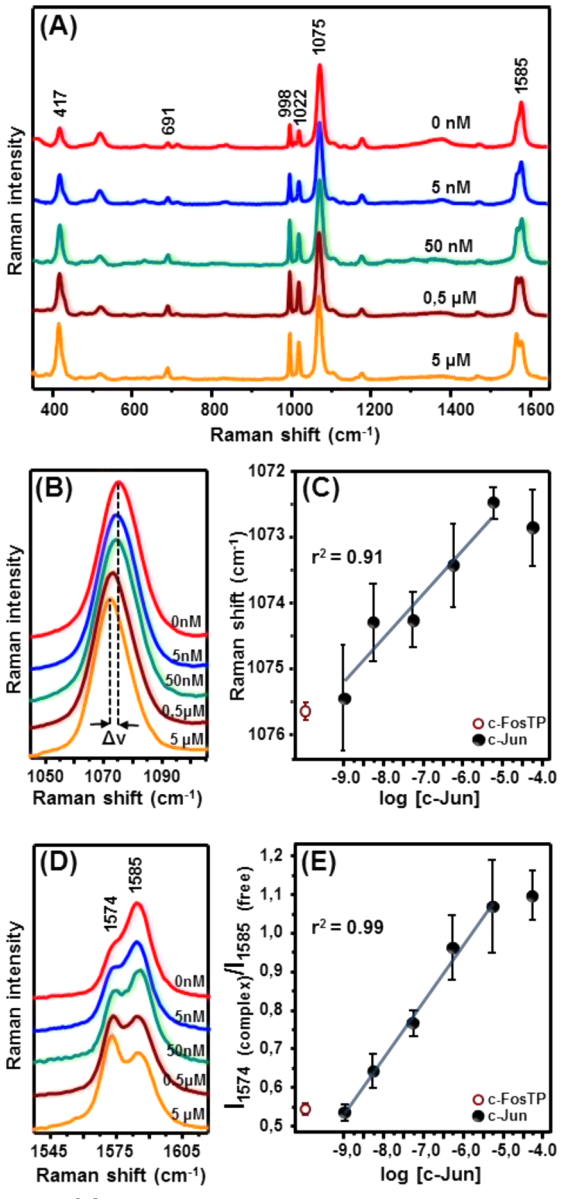

Figure 2. (A) Normalized SERS spectra of c-FosTP anchored to AgNPs over a silanized glass slide upon exposure to variable concentrations of c-Jun in HEPES buffer. (B,D) Details of the 1000−1100 and 1540−1620 cm^{-1} spectral regions of the SERS spectra illustrated in (A), respectively. (C) Spectral shift of the thiophenol band at ca. 1075 cm^{-1} as a function of c-Jun concentration (logarithmic scale) in HEPES buffer. (E) Intensity ratio $I_{1574(complex)}/I_{1585(free)}$ as a function of c-Jun concentration (logarithmic scale) in HEPES buffer, with a limit of detection at 5 nM. Error bars equal to two standard deviations (N = 3).

the benzene ring on the metal surface. The biomolecular recognition event can therefore be schematically summarized as depicted in Figure 1B; the protein c-FosTP exists in its unstructured monomeric form in the self-assembled monolayer. The presence of c-Jun promotes the formation of heterodimeric coiled-coil complexes and therefore a drastic restructuring of the geometry into a more rigid conformation. This structural change leads to a sensible deformation of the phenyl ring sandwiched in the c-Jun/c-Fos Ph−S−Ag surface complex, which is immediately revealed by the spectral analysis of the SERS data. The spectral changes illustrated in Figure 2B,D were correlated quantitatively with the c-Jun concentration using as spectral markers (i) the peak position of the ring breathing and ν(CS) feature (Figure 2C) and (ii) ratiometric peak intensities, $I_{1574(complex)}/I_{1585(free)}$ (Figure 2E), respectively. Spectral deconvolution was performed for each Raman band between 1574 and 1585 cm^{-1} at fixed fwhm values with the assumption of Lorentzian lineshapes.[38] Higher sensitivity to low amounts of c-Jun and better reproducibility from sample to sample are observed in the plot of $I_{1574(complex)}/I_{1585(free)}$ versus analyte concentration, which shows a detection limit of 5 nM and r^2 = 0.99 in the 1×10^{-9}−5×10^{-6} M range, with a saturation point at 5×10^{-6} M.

Importantly, c-Jun can also be clearly detected in cell extracts (total protein concentration equals to 10 times in weight the c-Jun concentration) albeit in this case the detection limit is approximately 1 order of magnitude higher, but still very good (DL = 50 nM, Figure 3). Careful optimization of the plasmonic

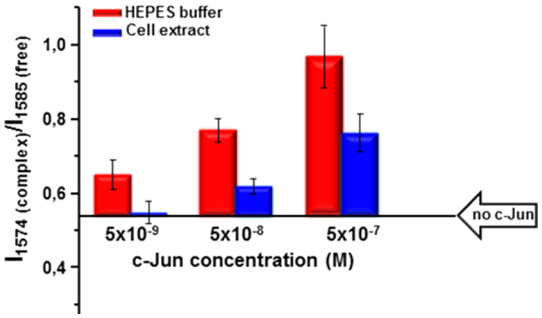

Figure 3. Intensity ratio $I_{1574(complex)}/I_{1585(free)}$ at different c-Jun concentrations in HEPES buffer and cell extracts. The X bar was placed at Y = 0.55 which corresponds to the intensity ratio value before the addition of c-Jun solutions. Error bars equal two standard deviations (N = 3).

substrate, such as the reduction of the exposed c-FosTP-modified metal surface area, or modification of the peptide receptor with acidic sequences to increase affinity,[17,39,40] should allow to further improving the sensitivity of the system. We also performed controls to assess the specificity of the SERS measurements. Interestingly, addition of HEPES buffer solutions of c-Fos (5×10^{-5} M), BSA (5×10^{-5} M), and cell extracts (380 μg/mL) left unaltered the SERS spectrum of c-FosTP, confirming the selectivity of the sensing strategy (Figure S3).

In summary, we have engineered a highly sensitive SERS-based sensor for the quantification of the oncoprotein c-Jun via functionalization of a nanostructured silver substrate with a c-Fos peptide receptor chemically modified by covalent attachment of a thiophenol group. Selective heterodimerization of the two proteins in the c-Fos/c-Jun complex triggers the molecular deformation of the TP structure, as revealed by characteristic changes in its SERS spectrum. Quantitative correlation between spectral marker bands and oncoprotein concentration allowed the detection of c-Jun down to the nanomolar regime in its complex biological medium. Even though it is demonstrated for the quantification c-Jun, potential applications of this sensing strategy may include other macromolecular analytes, thus giving rise to an extremely valuable tool in diagnosis and chemical biology.

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■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, peptide synthesis and labeling protocols, Raman spectra of proteins, analysis of SERS data, SERS spectra of control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Latchman, D. S. *Eukaryotic Transcription Factors*, 4th ed.; Academic Press: London, 2004; p 77.
(2) Maeda, S.; Karin, M. *Cancer Cell* **2003**, *3*, 102.
(3) Ma, Y. Q.; Li, Q. X.; Cui, W. L.; Miao, N.; Liu, X.; Zhang, W.; Zhang, C.; Wang, J. *Diagn. Pathol.* **2012**, *7*, 120.
(4) Blau, L.; Knirsh, R.; Ben-Dror, I.; Oren, S.; Kuphal, S.; Hau, P.; Proescholdt, M.; Bosserhoff, A. K.; Vardimon, L. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, E2875.
(5) Zhang, Y.; Pu, X. Y.; Shi, M.; Chen, L. Y.; Song, Y. H.; Qian, L.; Yuan, G. G.; Zhang, H.; Yu, M.; Hu, M. R.; Shen, B. F.; Guo, N. *BMC Cancer* **2007**, *7*, 145.
(6) Akatsu, Y.; Saikawa, Y.; Kubota, T.; Akatsu, T.; Yoshida, M.; Kitagawa, Y.; Otani, Y.; Kumai, K.; Kitajima, M. *Cancer Sci.* **2007**, *98*, 707.
(7) Eferl, R.; Ricci, R.; Kenner, L.; Zenz, R.; David, J. P.; Rath, M.; Wagner, E. F. *Cell* **2003**, *112*, 181.
(8) Wang, H. L.; Birkenbach, M.; Hart, J. *Carcinogenesis* **2000**, *21*, 1313.
(9) Szabo, E.; Riffe, M. E.; Steinberg, S. M.; Birrer, M. J.; Linnoila, R. I. *Cancer Res.* **1996**, *56*, 305.
(10) Smith, L. M.; Wise, S. C.; Hendricks, D. T.; Sabichi, A. L.; Bos, T.; Reddy, P.; Brown, P. H.; Birrer, M. J. *Oncogene* **1999**, *18*, 6063.
(11) Vogt, P. K. *Oncogene* **2001**, *20*, 2365.
(12) Garner, M. M.; Revzin, A. *Nucleic Acids Res.* **1981**, *9*, 3047.
(13) Galas, D. J.; Schmitz, A. *Nucleic Acids Res.* **1978**, *5*, 3157.
(14) Fang, Z. Y.; Zhang, W. J.; Ge, C. C.; Liu, J.; Lie, P. C.; Zeng, L. W. *Analyst* **2012**, *137*, 4127.
(15) Berger, M. F.; Bulyk, M. L. *Methods Mol. Biol. (Clifton, N.J.)* **2006**, *338*, 245.
(16) Zhu, C.; Byers, K.; McCord, R. P.; Shi, Z. W.; Berger, M. F.; Newburger, D. E.; Saulrieta, K.; Smith, Z.; Shah, M. V.; Radhakrishnan, M.; Philippakis, A. A.; Hu, Y. H.; De Masi, F.; Pacek, M.; Rolfs, A.; Murthy, T.; LaBaer, J.; Bulyk, M. L. *Genome Res.* **2009**, *19*, 556.
(17) Pazos, E.; Jimenez-Balsa, A.; Mascarenas, J. L.; Vazquez, M. E. *Chem. Sci.* **2011**, *2*, 1984. A similar appoach was later used for the development of folded RNA hairpins: Penas, C.; Pazos, E.; Mascareñas, J. L.; Vazquez, M. E. *J. Am. Chem. Soc.* **2013**, *135*, 3812.
(18) Bantz, K. C.; Meyer, A. F.; Wittenberg, N. J.; Im, H.; Kurtulus, O.; Lee, S. H.; Lindquist, N. C.; Oh, S. H.; Haynes, C. L. *Phys. Chem. Chem. Phys.* **2011**, *13*, 11551.
(19) Alvarez-Puebla, R. A.; Liz-Marzan, L. M. *Small* **2010**, *6*, 604.
(20) Ahijado-Guzmán, R.; Gómez-Puertas, P.; Alvarez-Puebla, R. A.; Rivas, G.; Liz-Marzán, L. M. *ACS Nano* **2012**, *6*, 7514.
(21) Moskovits, M. *Rev. Mod. Phys.* **1985**, *57*, 783.
(22) Aroca, R. *Surface-enhanced Vibrational Spectroscopy*; John Wiley & Sons: Chichester, 2006.
(23) Moskovits, M.; Suh, J. S. *J. Phys. Chem.* **1984**, *88*, 5526.
(24) Han, X. X.; Zhao, B.; Ozaki, Y. *Trac-Trends Anal. Chem.* **2012**, *38*, 67.
(25) Grubisha, D. S.; Lipert, R. J.; Park, H. Y.; Driskell, J.; Porter, M. D. *Anal. Chem.* **2003**, *75*, 5936.
(26) Wang, Y.; Wei, H.; Li, B.; Ren, W.; Guo, S.; Dong, S.; Wang, E. *Chem. Commun.* **2007**, *48*, 5220.
(27) Fabris, L.; Dante, M.; Nguyen, T. Q.; Tok, J. B. H.; Bazan, G. C. *Adv. Funct. Mater.* **2008**, *18*, 2518.
(28) Wang, Y. L.; Lee, K.; Irudayaraj, J. *Chem. Commun.* **2010**, *46*, 613.
(29) Kho, K. W.; Dinish, U. S.; Kumar, A.; Olivo, M. *ACS Nano* **2012**, *6*, 4892.
(30) Dyson, H. J.; Wright, P. E. *Nat. Rev. Mol. Cell. Biol.* **2005**, *6*, 197.
(31) Vinson, C.; Myakishev, M.; Acharya, A.; Mir, A. A.; Moll, J. R.; Bonovich, M. *Mol. Cell. Biol.* **2002**, *22*, 6321.
(32) Portela, C.; Albericio, F.; Eritja, R.; Castedo, L.; Mascareñas, J. L. *ChemBioChem* **2007**, *8*, 1110.
(33) Alvarez-Puebla, R. A.; Liz-Marzan, L. M. *Angew. Chem., Int. Ed.* **2012**, *51*, 11214.
(34) Jung, H. Y.; Park, Y.-K.; Park, S.; Kim, S. K. *Anal. Chim. Acta* **2007**, *602*, 236.
(35) Zayak, A. T.; Hu, Y. S.; Choo, H.; Bokor, J.; Cabrini, S.; Schuck, P. J.; Neaton, J. B. *Phys. Rev. Lett.* **2011**, *106*, 1357.
(36) Carron, K. T.; Hurley, L. G. *J. Phys. Chem.* **1991**, *95*, 9979.
(37) Saikin, S. K.; Olivares-Amaya, R.; Rappoport, D.; Stopa, M.; Aspuru-Guzik, A. *Phys. Chem. Chem. Phys.* **2009**, *11*, 9401.
(38) Thomas, G. J.; Agard, D. A. *Biophys. J.* **1984**, *46*, 763.
(39) Krylov, D.; Kasai, K.; Echlin, D. R.; Taparowsky, E. J.; Arnheiter, H.; Vinson, C. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12274.
(40) Olive, M.; Krylov, D.; Echlin, D. R.; Gardner, K.; Taparowsky, E.; Vinson, C. *J. Biol. Chem.* **1997**, *272*, 18586.