Evidence for a Coiled-coil Interaction Mode of Disordered Proteins from Bacterial Type III Secretion Systems*

Anastasia D. Gazi‡§, Marina Bastaki†§, Spyridoula N. Charova‡§, Eirini A. Gkougkoulia§1, Efthymios A. Kapellios§, Nicholas J. Panopoulos†§, and Michael Kokkinidis†§

Institute of Molecular Biology & Biotechnology, Foundation of Research & Technology, and the Department of Biology, University of Crete, Vasilika Vouton, GR 71110 Heraklion, Crete, Greece.

Type III secretion systems (T3SS) are essential mediators of the interaction of many Gram-negative pathogenic proteobacteria (α, β, γ, and δ subdivisions) with their human, animal, or plant hosts and are evolutionarily related to bacterial flagella (1–3). They act as multiprotein nanomachines (injectosomes) that translocate a diverse repertoire of proteins (effectors) either to extracellular locations or directly into eukaryotic cells, in a Sec-independent manner (interkingdom protein transfer). The effectors modulate the function of crucial host regulatory molecules and trigger a range of highly dynamic cellular responses, which determine pathogen-host recognition, pathogen/symbiont accommodation and elicitation or suppression of defense responses by the eukaryotic hosts. T3SS have evolved into seven families distributed among Gram-negative proteobacteria by horizontal gene transfer (3).

The core of the T3SS apparatus is formed by a set of nine widely conserved proteins that share sequence and structural homologies that span the divide between flagellar and non-flagellar systems, as evidenced by the structural similarities between the homologous domains of HrcQα (4) and FliN (5), or the ATPases EscN (6) and FliI (7). On the other hand, despite considerable sequence diversity (2), many secreted T3SS components, exhibit sequence motifs strongly favoring coiled-coil formation, a property that they share with effectors and numerous other proteins involved in translocation and secretion regulation (8, 9).

The coiled-coil tertiary motif in proteins consists of 2–5 amphipathic α-helices winding around each other to form a supercoil structure and is associated with specific sequence patterns (heptad repeats) that reflect repeating variations of physicochemical properties (10, 11). The motif is highly versatile, serving a broad range of functions, and occurring both in monomeric form and a variety of oligomeric assemblies; its prevalence in T3SS suggests that it may contribute to fundamental requirements of their function in a manner that is not yet understood. In various biological systems, coiled-coils are frequently unfolded as monomers and fold only upon association and formation of quaternary complexes (12), while coiled-coil structures from early folding intermediates are frequently essential for molecular recognition (13, 14).

In this study the in vitro folding of HrpO, a soluble protein from the T3SS of the plant pathogen Pseudomonas syringae pv phaseolica (15) will be presented, along with its interactions and relations to other T3SS proteins. Our interest in HrpO was provoked by recent insights into the function of soluble components of the export apparatus (16, 17), and in particular by its analogies to the flagellar export protein FliJ that has an essential function at the level of the export ATPase complex (18). HrpO and FliJ have similar size, are encoded by genes located immediately downstream of the atpase gene of their respective gene clusters and despite the absence of significant sequence homol-

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‡ Recipient of an Onassis Foundation fellowship.
§ To whom correspondence should be addressed: Institute of Molecular Biology & Biotechnology, Foundation of Research & Technology, Vasilika Vouton, GR 71110 Heraklion, Crete, Greece. Tel. and Fax: 3028810394351; E-mail: kokkinidl@imbb.forth.gr.

The abbreviations used are: T3SS, type III secretion system; SAXS, small angle X-ray scattering; CAPS, 3-cyclohexylamino)propanesulfonic acid; NTA, nitrilotriacetic acid; PDB, Protein Data Bank.
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Cloning, Expression, and Purification—N-terminally His6-tagged HrpO was expressed in *Escherichia coli* DH5α cells and purified using a Ni-NTA-agarose column and a Sephacryl S-100 gel filtration column. C-terminally His6-tagged HrpE was expressed in *E. coli* BL21(DE3) cells and purified using a Ni-NTA-agarose column (see supplemental data for details).

**Gel Filtration Analysis**—Gel filtration was performed at 20 °C using an ÁKTA purifier system (Amersham Biosciences) with a calibrated XK16/100 column packed with Sephacryl S100. The flow rate was 0.8 ml/min, and elution was monitored at 280 nm. Sample solutions were prepared to a concentration of 5–30 mg/ml and loaded using a 2-ml loop.

**Small Angle X-ray Scattering (SAXS)**—Data were collected at 4 °C for three different HrpO concentrations (1.8, 3.6, and 7.3 mg/ml) at beamline X33 at EMBL/DESY using a MAR345 detector (21) at a sample-detector distance of ~3 m (covering a momentum transfer range of 0.08 < q < 0.45 Å⁻¹), mica cells with 1-mm path length, and a collection time of 2 min per frame (21). Using PRIMUS (22) the data were analyzed for radiation damage, averaged after normalization to the intensity of the incident beam, and corrected for the detector response. PRIMUS was also used for the buffer subtraction, data scaling, calculation of the radius of gyration \( R_g \) from the slope of the Guinier plot (\( \ln(I(s)) \) versus \( s^2 \)) (23) and the radius of gyration of the cross section (\( R_{gC}s \)) (24), based on the Guinier approximation for a rod particle (scattering intensity: \( I(\theta) \); momentum transfer: \( s = 4\pi\sin(\theta)/\lambda \); 2\( \theta \): scattering angle; \( \lambda = 1.5 \) Å: wavelength of x-rays). The forward scattering intensity \( I(0) \) (proportional to the number of electrons of the particle) was estimated through extrapolation of the Guinier plot at 29 = 0° and used to deduce molecular mass of HrpO in solution. MW calibration was based on scattering data from a 5 mg/ml bovine serum albumin solution in 20 mM HEPES, pH 7.5. The GNOM package (25) was used to calculate the pair distribution function \( P(r) \) and to estimate the maximum particle dimension \( (l_{\text{max}}) \). Kratyk plots were obtained by plotting \( I(s) \times s^2 \) versus \( s \) and used to judge the folding of the protein.

**EOM** (26), was used to quantitatively characterize the disorder of HrpO by allowing for the coexistence of different conformations contributing to the experimental scattering pattern. These conformers were selected using a genetic algorithm (GAJOE) from a pool containing 10,000 models randomly generated by RanCh (26) to cover the conformational space.

**Circular Dichroism**—CD spectra were acquired using a J-815 CD spectropolarimeter (Jasco Inc., Easton, MD) with a 1-mm path length quartz cuvette, at HrpO concentrations of 0.02–0.21 mg/ml, 20 mM phosphate buffer, pH 8.5, 50 mM KF. Far-UV spectra were measured with 50 nm/min scanning speed, 1 min response time, and 3 accumulations. Far-UV CD spectra were also collected for the HrpE protein at concentrations 0.16 and 0.33 mg/ml in 20 mM phosphate buffer, pH 8.5, 50 mM KF. The Spectra Manager program (Jasco Corp.) was used for buffer subtraction and unit conversions to molar residual ellipticities. Thermal denaturation data were collected monitoring the CD signal at 222 nm in the range of 0–90 °C with a temperature increase of 80 °C/h and a waiting time of 3 s for stabilization. Full far-UV CD spectra (260–195 nm) were also recorded in the range of 4–64 °C during the above procedure in steps of 4 °C. Singular Value Decomposition (SVD) (27) of the thermal denaturation spectra using the program SVD was performed to determine significant independent states in the unfolding transition. The significant species needed to model the CD data as a function of temperature were determined on the basis of the characteristics of the SVD basis vectors \( u_i(\theta) \) and the temperature dependence of their associated coefficients \( v_i \). The thermal unfolding curve was fitted using SigmaPlot, assuming a two-state model \( (N \rightarrow U) \) (28). Far-UV CD spectra were also recorded at 4 °C after the sample was heated at 90 °C to verify the reversibility of the thermal unfolding transition.

**Yeast Two-hybrid System**—Yeast two-hybrid assay was performed using MATCHMAKER Two-hybrid System 2 (Clontech). The HrpO gene was inserted into the pAS2-1 plasmid to create an in-frame fusion with the GAL4 DNA-binding domain. The HrpE gene was cloned in the pACT2 plasmid and fused in frame with the transcription activation domain of GAL4. The plasmids were transformed into *Saccharomyces cerevisiae* strain PJ6-4A carrying three different GAL4-responsive reporter genes (ade, his, and lacZ), each driven by a different GAL4-responsive promoter, to reduce the incidence of false positives. The interaction of hybrid proteins was examined by growing the transformed cells on complete minimal medium lacking Trp, Leu, Ade, and His for 3 days at 30 °C. Plasmids pVA3-1 and pTD1-1 are DNA-binding domain and activation domain fusion plasmids that provide a positive control for interacting proteins.

**Co-purification of HrpE and HrpO**—Co-expressed His10-HrpE and HrpO were co-purified by metal affinity chromatography from *E. coli* BL21(DE3) cells co-expressing His10-HrpE and HrpO. Details are given in supplemental data.

**Sequence Analysis**—T3SS gene clusters were retrieved from GenBankTM. For all T3SS families products of genes located immediately downstream the T3SS *atpase* gene, and having a comparable size to HrpO were analyzed for the detection of intrinsic unfolding (program FoldIndex, Ref. 29), coiled-coil propensities (COILS (11) and MATCHER (30)) and secondary structure (PSIPRED (31)). Three-dimensional structural profile recognition from sequences was performed with PHYRE (32). Homologues of HrpE were found using PSI-BLAST. Protein physicochemical parameters were calculated with ProtParam (33).

**RESULTS**

HrpO Forms Heterogeneous Populations of Oligomers in Vitro—When subjected to size-exclusion chromatography at high protein concentrations, the 147-residue HrpO protein elutes with an asymmetric peak profile at an apparent molecular weight
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(MW) of ~51 kDa (Fig. 1A), corresponding either to a dimer or trimer (assuming globularity) or to a highly extended non-globular conformation. The broadness of the peak indicates polydispersity. At lower concentrations the elution profiles shifts toward smaller apparent MW.

SAXS was used to analyze the HrpO oligomerization. From the forward scattering intensity I(0) a MW of ~23 kDa was determined at low concentrations (~2 mg/ml) suggesting a prevalence of the monomer form (supplemental Table S1). The I(0) values are concentration-dependent (Fig. 1B) suggesting self-association effects. At 7 mg/ml the average MW is ~77 kDa, corresponding to tetramers or higher order oligomers. Guinier plots of the scattering curves (Fig. 1C) show increasing deviations from linearity at higher concentrations, an indication of polydisperse oligomer populations and of self-association toward complexes of increasingly higher average size (Fig. 1C).

HrpO Is Highly α-Helical and Undergoes a Two-state Thermal Unfolding with Very Low Melting Temperature—Far-UV CD spectra of HrpO show the characteristic minima of α-helical proteins at 208 and 222 nm (Fig. 2). From the mean residual ellipticity at 222 nm, an α-helical content of ~60% is estimated (34) at 4 °C, which decreases to ~50% at 20 °C and to ~20% at 60 °C (HrpO concentration, 0.1 mg/ml). The α-helical content is concentration dependent and increases by ~50% when concentration increases from 0.02 to 0.1 mg/ml (supplemental Fig. S1). An increase of the ionic strength of the solution from 25 to 250 mM Na2SO4 also increases α-helical content by ~50% (Fig. 2D).

The thermal unfolding of HrpO was monitored by the temperature dependence of the CD signal at 222 nm (Fig. 2A). The 1st derivative of the thermal unfolding data reveals an unusually low transition temperature of 21 °C. Fitting the thermal unfolding data by a curve under the assumption of a two-state transition (Fig. 2A, red line), yields a melting temperature of 21 ± 4 °C, in good agreement with the value obtained by the 1st
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This study shows that the HrpO protein forms low to high order oligomers at different concentrations. It is highly α-helical with coiled-coil characteristics, very low melting temperature, and does not possess a well folded structure. HrpO interacts with HrpE, another α-helical T3SS protein that is predicted to possess coiled-coils. This interaction was identified through yeast two-hybrid data and confirmed by co-purification. Sequence analysis suggests that HrpO has characteristics of an intrinsically disordered coiled-coil with extensive analogies to FliJ and to several other proteins across all T3SS families, despite low levels of sequence similarities.

Proteins span a continuum from totally disordered to well folded structures. The extreme flexibility of intrinsically disordered proteins, whose structures may alter dramatically as they bind to their cognate folded protein target, has been suggested to represent a strategy for optimizing the search and interaction with their targets (47). This strategy may also apply to the binding of HrpO to its protein targets, as suggested by its pronounced flexibility and the observed α-helical ordering effects associated with the HrpO/HrpE interaction. The observed properties of HrpO and the predicted properties of its analogues from other T3SS, suggest that HrpO represents a class of intrinsically disordered proteins present in all T3SS families and the flagellum that bind target molecules (e.g. HrpE) probably via coiled-coil interactions. The pronounced self-association propensity of HrpO that produces quaternary complexes in which coiled-coil interactions play a role, as suggested by CD spectra, has probably analogies to the interactions of HrpO with its coiled-coil target proteins. Intrinsic disorder is reflected in the double wavelength plot (Fig. 2E) of [θ]_{200} versus [θ]_{222} (39), which reveals that in the temperature range 4–20 °C HrpO exists at the borderline between a folded (coiled-coil) and a molten globule-like state, with more features of intrinsic disorder (molten globule and pre-molten globule states) being gradually established beyond 20 °C. Thermal unfolding studies reveal a two-state transition, with a strikingly low transition temperature of ~21 °C. SAXS data suggest that at low concentrations HrpO exists as an ensemble of disordered, highly α-helical conformers characterized by lack of globularity, although it is not completely unfolded. We believe that at concentrations favoring the monomeric form, HrpO contains a core formed by some secondary structure elements, with prevailing coiled-coil interactions, as evidenced by the characteristics of CD spectra. The remainder of the polypeptide chain is disordered. The lack of globularity i.e. of a tightly packed core, deduced from the Kratky plot at low concentrations, indicates that this core is probably small and loosely packed. More globular features are induced at higher concentrations, probably due to association effects producing higher order multimers, as evidenced by the Kratky plot and I(0). Presumably, either the association of monomers occurs first leading to the induction of additional structure, or under the specific conditions, the population of more structured intermediates increases, which then associate. It is likely that the association of disordered intermediates occurs via coiled-coil interactions, thereby stabilizing partial secondary structural elements and leading to the formation of additional helices, as observed by CD. Some analogies exist with the process via which α-helical peptides stabilize their helical structures upon association at high concentrations (48), or with the association-induced folding of partially folded intermediates of staphylococcal nuclease (49). In contrast to the above cases however, only limited additional globular characteristics are induced upon increase of the HrpO α-helical content due to oligomerization.

The properties of HrpO at even relatively low temperatures resemble those of a molten globule state although it is not clear how relevant they are physiologically. Intrinsic disorder and the coiled-coil interactions may play an important role in the recognition of HrpO binding partners in T3SS, where coiled-coils containing proteins (e.g. HrpE) are abundant. A more structured state of HrpO with specific contacts may be established upon association to its partner, similarly to what we observe in HrpO oligomerization and in the HrpO-HrpE interaction. This process could be also comparable to the interaction of the...
intrinsically disordered pKID domain of gene transcription factor CREB with the KIX domain of the CREB-binding protein in the cell nucleus (47). Conformational flexibility and coiled-coil interactions were recently confirmed as necessary requirements for complex formation in the case of tropomyosin-actin binding (13).

Generally, intrinsic disorder is a distinctive and common characteristic of functional interactions (50), and more specifically of hub proteins (proteins that interact with more than 10 partners) in eukaryotic interactomes (51). Disorder could be thus a determinant of functional interactivity of HrpO and its analogues from other T3SS gene clusters (supplemental Table S2) and provide a basis for a widespread coiled-coil interaction mode between proteins of the HrpO family and their coiled-coil targets. This possibility is supported by the properties of the HrpO target HrpE that is α-helical and is predicted to form extensive coiled-coil regions. These regions could be involved at the stage of the initial encounter complex between HrpO and HrpE and facilitate the transition toward more structured states/α-helical ordering, in agreement with the finding that α-helical molecular recognition elements (interaction-prone short segments of disorder that become ordered upon specific binding) are abundant in protein-protein interaction networks (52). HrpE homologues with coiled-coil propensity are found for nearly every T3SS in which HrpO-analogues have been identified (supplemental Table S3).

The structures of the interaction partners of FliJ, the flagellum counterpart of HrpO, also support the concept of a coiled-coil interaction mode: FliJ interacts with FliH, a HrpE homologue, for which high α-helical and coiled-coil segments are predicted. In addition, FliJ interacts directly with the flagellar export chaperone FlgN (18), a 4-α-helical bundle (PDB ID: 2fup). A 20-residue coiled-coil region of FlgN has been mapped to be the common binding site for FliJ and for the export substrate FlgK (PDB ID: 2d4y) the structure of which has as its most prominent feature a coiled-coil domain comprising ~50% of all residues. The association of FliJ or FlgK to their common binding site is thus probably mediated through interactions between coiled-coil segments.

If the properties of HrpO can be projected to FliJ (an assumption that is supported by the characteristics of the FliJ protein (19)), then the properties of intrinsic disorder and coiled-coil propensity may be also understood under the aspect of the regulation of the flagellar T3SS export apparatus: At the level of the export ATPase complex, two activities have been reported for FliJ, i.e. a T3SS chaperone escort activity and a stimulation of the FliJ ATPase activity (18). The escort mechanism for export chaperones (FlgN, Flt) clears the membrane FliJ ATPase complex prior to unfolding and translocation of the export substrate that is driven proton motive force (17).

FliJ is thus probably involved in T3S regulation through two activities at the ATPase complex, and these require interactions with several coiled-coil proteins of different structures. Coiled-coil propensity and a considerable structural plasticity, reflected in intrinsic structural disorder, are thus essential prerequisites for FliJ function. It should be noted that FliJ also interacts with further T3SS components, e.g. the C-ring protein FlIM (20), which also exhibits coiled-coil regions, so that this pronounced interactivity is probably enabled by a considerable structural disorder (19).

In summary, the observed properties of HrpO, the properties of the FliJ interactions network, a large body of sequence and structural data and the prevalence of the coiled-coil motif, support the hypothesis of the existence of a widespread coiled-coil interaction mode in all T3SS families and the flagellum mediated by intrinsically disordered proteins. The functional implications of this interaction mode are not clear yet; however they may include a role in the regulation of the T3SS export apparatus.

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REFERENCES

1. Dale, C., and Moran, N. A. (2006) Cell 126, 453–465
2. Tampakaki, A. P., Fadouloglou, V. E., Gazi, A. D., Panopoulos, N. J., and Kokkinidis, M. (2004) Cell. Microbiol. 6, 805–816
3. Troisfontaines, P., and Cornелиs, G. R. (2005) Physiology 20, 326–339
4. Fadouloglou, V. E., Tampakaki, A. P., Glykos, N. M., Bastaki, M. N., Hadjen, J. M., Phillips, S. E., Panopoulos, N. J., and Kokkinidis, M. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 70–75
5. Brown, P. N., Mathews, M. A. A., Joss, L. A., Hill, C. P., and Blair, D. F. (2005) J. Bacteriol. 187, 2890–2902
6. Zarivach, R., Vuckovic, M., Deng, W., Finlay, B. B., and Strynadka, N. C. J. (2007) Nat. Struct. Mol. Biol. 14, 131–137
7. Imada, K., Minamino, T., Tahara, A., and Namba, K. (2007) Proc. Natl. Acad. Sci. U. S. A. 104, 485–490
8. Delahay, R. M., and Frankel, G. (2002) Mol. Microbiol. 45, 905–916
9. Pallen, M. J., Beatson, S. A., and Bailey, C. M. (2005) FEMS Microbiol. Rev. 29, 201–229
10. Paliakasis, C. D., and Kokkinidis, M. (1992) Prot. Engineering 5, 739–749
11. Lupas, A., Van Dyke, M., and Stock, J. (1991) Science 252, 1162–1164
12. Uversky, V. N., Gillespie, J. R., and Fink, A. L. (2000) Proteins Struct. Func. Genet. 41, 415–427
13. Singh, A., and Hitchcock-DeGregori, S. E. (2006) Structure 14, 43–50
14. Steinmetz, M. O., Jelezarov, I., Matousek, W. M., Honnapa, S., Jahne, W., Missimer, J. H., Frank, S., Alexandrescu, A. T., and Kammerer, R. A. (2007) Proc. Natl. Acad. Sci. U. S. A. 104, 7062–7067
15. Lindgren, P. B., Peet, R. C., and Panopoulos, N. J. (1986) J. Bacteriol. 168, 512–522
16. Paul, K., Erhardt, M., Hirano, T., Blair, D., and Hughes, K. T. (2008) Nature 451, 489–493
17. Minamino, T., and Namba, K. (2008) Nature 451, 485–489
18. Evans, L. D. B., Stafford, G. P., Ahmed, S., Fraser, G. M., and Hughes, C. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 17474–17479
19. Fraser, G. M., Gonzalez-Pedrajo, B., Tame, J. R. H., and Macnab, R. M. (2003) J. Bacteriol. 185, 5546–5554
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Mol. Microbiol. 60, 984–998
21. Roessle, M. W., Klaering, R., Ristau, U., Robrah, B., Jahn, D., Gehrmann, T., Konarev, P., Round, A., Fiedler, S., Hermes, C., and Svergun, D. (2007) J. Appl. Crystallogr. 40, s190–s194
22. Konarev, P. V., Volkov, V. V., Sokolova, A. V., Koch, M. H. J., and Svergun, D. I. (2003) J. Appl. Crystallogr. 36, 1277–1282
23. Guinier, A., and Fournet, G. (1955) Small-Angle X-ray Scattering of X-rays, Wiley, New York
24. Ishimuro, Y., Hamada, F., and Nakajima, A. (1979) J. Polym. Sci. 17, 1811–1819
25. Svergun, D. I. (1993) J. Appl. Crystallogr. 26, 258–267
26. Bernadó, P., Mylonas, E., Petoukhov, M. V., Blackledge, M., and Svergun, D. I. (2007) J. Am. Chem. Soc. 129, 5656–5664
27. Pérez, J., Vachette, P., Russo, D., Desmadril, M., and Durand, D. (2001) J. Mol. Biol. 308, 721–743
28. Greenfield, N. J. (2006) Nat. Protoc. 1, 2527–2535
29. Prilusky, J., Felder, C. E., Zeev-Ben Mordehai, T., Rydberg, E. H., Man, O., Beckmann, J. S., Silman, I., and J. L., S. (2005) Bioinformatics 21, 3435–3438
30. Fischetti, V. A., Landau, G. M., Schmidt, J. P., and Sellers, P. (1993) Inform. Process Lett. 45, 11–18
31. Jones, D. T. (1999) J. Mol. Biol. 292, 195–202
32. Kelley, L. A., MacCallum, R. M., and Sternberg, M. J. E. (2000) J. Mol. Biol. 299, 499–500
33. Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., and Bairoch, A. (2005) The Proteomics Protocols Handbook, Humana Press
34. Chen, Y.-H., Yang, J. T., and Chau, K. H. (1974) Biochemistry 13, 3350–3359
35. Holtzer, M. E., and Holtzer, A. (1995) Biopolymers 36, 365–379
36. Wall, M. E., Rechtsteiner, A., and Rocha, L. M. (2003) Singular Value Decomposition and Principal Component Analysis, Kluwer, Norwell
37. Lees, J. G., Miles, A. J., Wien, F., and Wallace, B. A. (2006) Bioinformatics 22, 1955–1962
38. Uversky, V. N. (2002) FEBS J. 269, 2–12
39. Uversky, V. N. (2003) Cell Mol. Life Sci. 60, 1852–1871
40. Hagihara, Y., Hoshino, M., Hamada, D., Kataoka, M., and Goto, Y. (1998) Folding Des. 3, 195–201
41. Jha, A. K., Colubri, A., Freed, K. F., and Sosnick, T. R. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 13099–13104
42. Dunker, A. K., Brown, C. J., Lawson, J. D., Iakoucheva, L. M., and Obradovic, Z. (2002) Prot. Funct. Biochem. 41, 6573–6582
43. Ramos, A. R., Morello, J. E., Ravindran, S., Deng, W.-L., Huang, H.-C., and Collmer, A. (2007) J. Bacteriol. 189, 5773–5778
44. Wall, M. E., Rechtsteiner, A., and Rocha, L. M. (2003) Singular Value Decomposition and Principal Component Analysis, Kluwer, Norwell
45. Sugase, K., Dyson, H. J., and Wright, P. E. (2007) Nature 447, 1021–1025
46. Yoshida, K., Shibata, T., Masai, J., Sato, K., Noguti, T., Go, M., and Yana-gawa, H. (1993) Biochemistry 32, 2162–2166
47. Uversky, V. N., Karnoup, A. S., Khurana, R., Segel, D. J., Doniach, S., and Fink, A. L. (1999) Protein Sci. 8, 161–173
48. Dyson, H. J., and Wright, P. E. (2005) Nat. Rev. Mol. Cell Biol. 6, 197–208
49. Haynes, C., Oldfield, C. J., Ji, F., Klitgord, N., Cusick, M. E., Radivojac, P., Uversky, V. N., Vidal, M., and Iakoucheva, L. M. (2006) PLoS Comp. Biol. 2, 890–901
50. Dunker, A. K., Cortese, M. S., Romero, P., Iakoucheva, L. M., and Uversky, V. N. (2005) FEBS J. 272, 5129–5148
51. Matsuo, K., Yonehara, R., and Gekko, K. (2004) J. Biochem. 135, 405–411
52. Vlassi, M., Steif, C., Weber, P., Tsernoglou, D., Wilson, K. S., Hinz, H.-J., and Kokkinidis, M. (1994) Nat. Struct. Biol. 1, 706–716
53. Mason, J. M., Schmitz, M. A., Müller, K. M., and Arndt, K. M. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 8989–8994
54. Holtzer, M. E., Braswell, E., Angeletti, R. H., Mints, L., Zhu, D., and Holtzer, A. (2000) Biophys. J. 78, 2037–2048
55. Dutta, K., Alexandrov, A., Huang, H., and Pascal, S. M. (2001) Protein Sci. 10, 2531–2540