

Supplementary Information

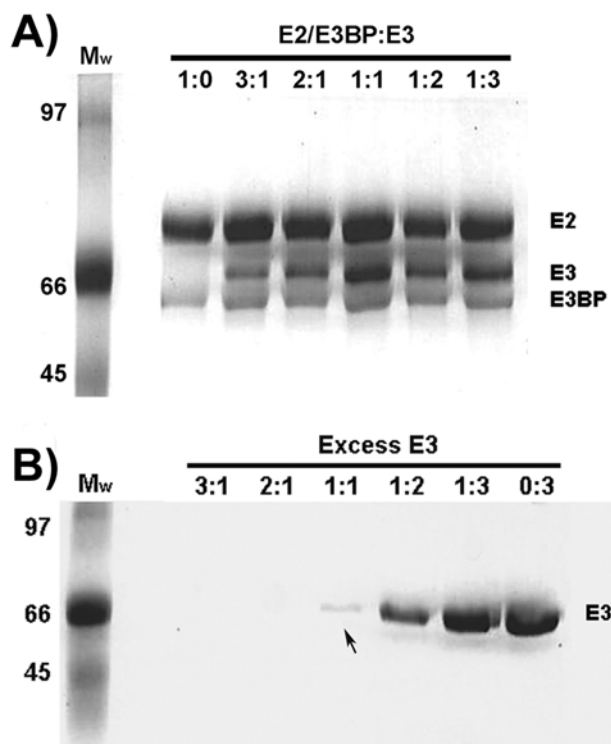


Figure S1 SDS-PAGE analysis of rhE2/E3BP:rhE3 stoichiometry

Peak fractions from GFC separation of rhE2/E3BP:rhE3 at a range of stoichiometries were analysed by SDS-PAGE. **(A)** Void volume (38 ml) GFC peak fractions confirm the incorporation of rhE3 in the complex. **(B)** GFC peak fractions collected at an elution volume of 65 ml show the appearance of excess rhE3 from a 1:1 ratio onwards (arrow). Molecular weights (Mw) are in kDa.

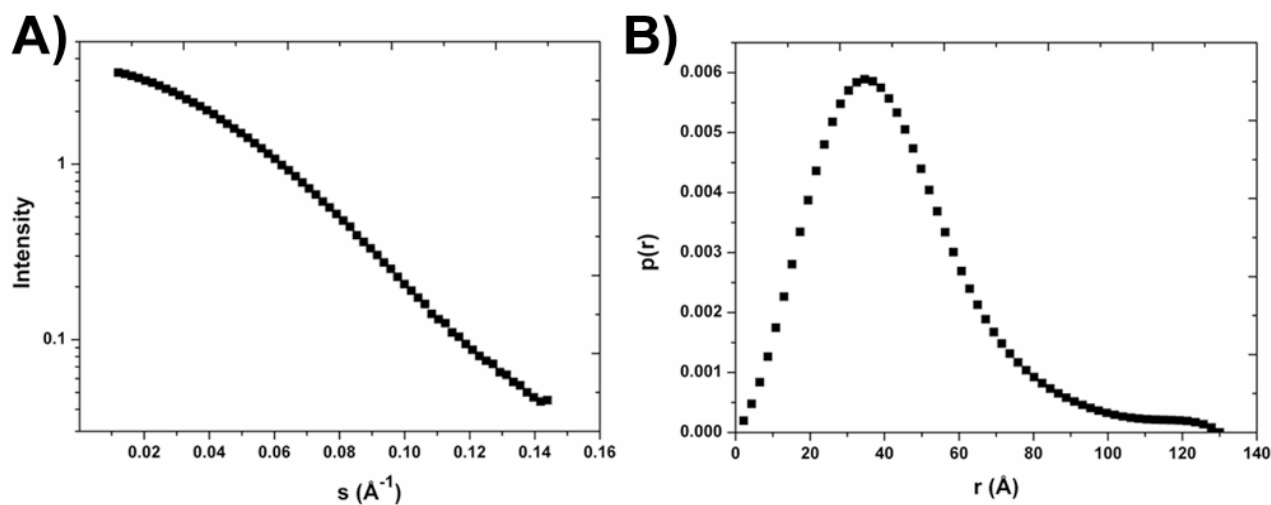


Figure S2 Small angle neutron scattering from deuterated dE3

(A) The SANS curve of uncomplexed dE3. **(B)** The distance distribution function, $p(r)$ calculated using GNOM yielded a D_{max} of 130 Å.

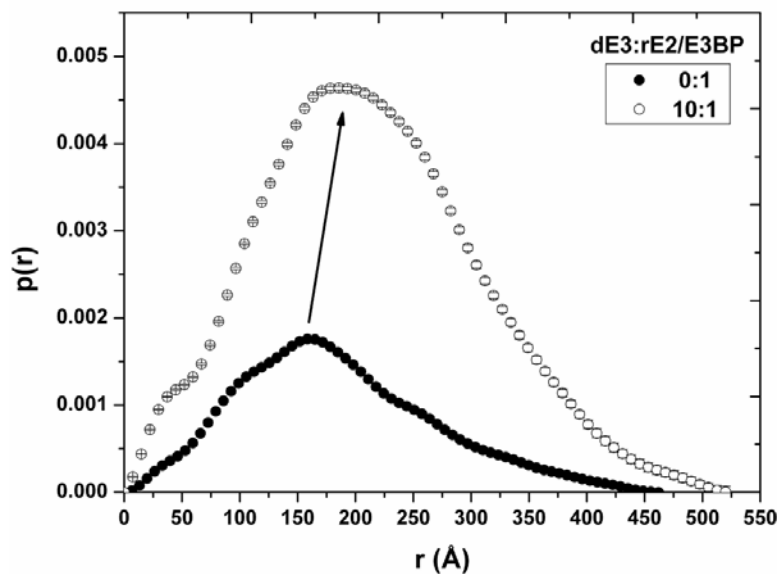


Figure S3 Distance distribution $p(r)$ analysis of dE3:rhE2/E3BP at 10:1 saturation

The $p(r)$ distributions were calculated using GNOM for uncomplexed rhE2/E3BP and dE3:rhE2/E3BP complex at a binding saturation ratio of 10:1. The radial shift to higher r upon dE3 binding to the core is denoted by an arrow. Error bars are shown but are not clearly visible due to their small size.

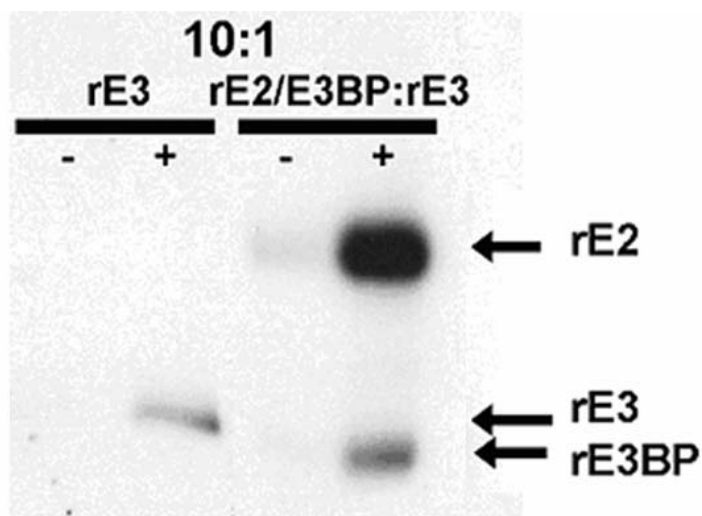


Figure S4 [^{14}C]-NEM fluorography of recombinant PDC core

Specific [^{14}C]-NEM incorporation into E2 and E3BP subunits of rhE2/E3BP core complexed with rhE3 at a molar ratio of 10:1 in the presence of NAD (-) and NADH (+) is denoted. An equivalent amount of uncomplexed rhE3 radiolabelled in the presence of NAD $^+$ (-) or NADH (+) was included as a control.

Tables

E3BP:E3 stoichiometry		
2:1	1:1	
Number of E3 dimers per rhE2/E3BP core		Core composition
10*	20	40E2+20E3BP
6	12	48E2+12E3BP

Table S1 Variable substitution rhE2/E3BP core models

* The number of dE3 required for saturation, as determined in the SANS stoichiometry study.

Core organisation	No. of homotrimers	No. of heterotrimers
58E2+2E3BP	18	2
56E2+4E3BP	16	4
54E2+6E3BP	14	6
52E2+8E3BP	12	8
50E2+10E3BP	10	10
48E2+12E3BP	8	12
46E2+14E3BP	6	14
44E2+16E3BP	4	16
42E2+18E3BP	2	18
40E2+20E3BP	0	20

Table S2 Plausible E2/E3BP core organizations and corresponding numbers of homo and hetero-trimers