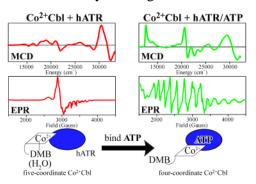
## A New Paradigm in Biological Co–C Bond Formation: Spectroscopic Studies of Human ATP:Cobalamin Adenosyltransferase

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In humans, exogenously derived vitamin  $B_{12}$  must be adenosylated before it can serve as the cofactor for the enzyme methylmalonyl-CoA mutase (MMCM). Malfunction of the human adenosyltransferase (hATR) that catalyzes this conversion can result in harmful accumulation of methylmalonyl-CoA, leading to the potentially fatal disease methylmalonic aciduria. Formation of the adenosylcobalamin (AdoCbl) cofactor is achieved by transferring the adenosyl group from co-substrate ATP to a transiently formed  $Co^{1+}$ cobalamin ( $Co^{1+}$ Cbl) species. A particularly puzzling aspect of hATR function is that the midpoint potential for  $Co^{2+}$ — $Co^{1+}$  reduction of the free Cbl cofactor is below that of readily available biological reductants suggesting that the redox active orbitals of  $Co^{2+}$ Cbl are significantly perturbed by hATR. To probe potential changes in the  $Co^{2+}$ Cbl geometric and electronic structures caused by binding to hATR in the

absence and presence of the co-substrate ATP we have employed magnetic circular dichroism (MCD) and EPR spectroscopies. Our data (see figure), interpreted within the theoretical framework established in our earlier work on free Co<sup>2+</sup>corrinoids, reveal that in the absence of ATP, the interaction between Co<sup>2+</sup>Cbl and hATR promotes partial conversion of the cofactor to its "base-off" form in which a water molecule occupies the lower axial position. This protein-induced perturbation of the cofactor becomes much stronger in the presence of



ATP, leading to the formation of an unprecedented Co<sup>2+</sup>Cbl species with spectroscopic signatures consistent with an essentially four-coordinate, square planar Co<sup>2+</sup> center. This unusual Co<sup>2+</sup>Cbl coordination is expected to raise the Co<sup>2+/1+</sup> reduction potential well into the physiological range. Interestingly, our preliminary data suggested that formation of this activated four-coordinate Co<sup>2+</sup>Cbl may be universal to all enzymes that catalyze Co–C bond formation.